PATENT
Attorney Docket No. MEE-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Adamis *et al.*	
SERIAL NO.:	09/478,099	GROUP NO.: 1632
FILED:	January 5, 2000	EXAMINER: Anne-Marie Baker
TITLE	*TARGETED TRANSSCLERAL CONTROLLED RELEASE DRUG DELIVERY TO THE RETINA AND CHOROID*	

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF KAREN G. CARRASQUILLO, PH.D.
UNDER 37 CFR 1.132

Sir:

I, Karen G. Carrasquillo, Ph.D. hereby declare as follows:

1. During the time period from Feb 2001 to June 2002, I was employed as a Research Fellow in the laboratory of Dr. Anthony P. Adamis at the Massachusetts Eye and Ear Infirmary, Boston, Massachusetts ("MEEI").

2. During the period of my employment at the MEEI, I conducted research on, among other things, the transcleral delivery of an anti-Vascular Endothelial Growth Factor (VEGF) aptamer known as EYE001 into a mammalian eye. Some of my research at the MEEI was published in a paper entitled "Controlled Delivery of the Anti-VEGF Aptamer EYE001 with Poly(lactic-co-glycolic) Acid Microspheres" in Carrasquillo *et al.* (2003) Invest. Ophthalmol. Vis. Res. 44: 290-299, a copy of which is attached behind Tab A.

3. I understand that Anthony P. Adamis, M.D., Evangelos S. Gragoudas, M.D., and Joan W. Miller, M.D. are named as co-inventors of the subject matter being claimed in the above-

identified patent application. I understand that the MEEI is owner of this patent application.

4. I am familiar with the pending patent application, the pending claims, and the outstanding Office Action. I am providing the following information about an *in vivo* study I performed while at the MEEI that demonstrates that a nucleic acid molecule known as EYE-001 - an anti-VEGF aptamer - can traverse the sclera and impart a biological effect in a mammalian eye, for example, reduce leakage from blood vessels in the interior of a mammalian eye.

5. Materials and Methods

5.1. EYE-001 aptamer was encapsulated within poly(lactic-co-glycolic) acid (PLGA) microspheres using an oil-in-oil solvent evaporation process. Briefly, 25-30 mg of lyophilized EYE001 were suspended by homogenization in a 2 mL solution of PLGA (200 mg) dissolved in methylene chloride. Two mL of the coacervating agent poly(dimethylsiloxane) were added to the suspension at a rate of 2 mL/min and homogenized for 1 minute at a rate 2,000 rpm. The resulting oil-in-oil suspension was added to 50 mL of heptane under constant agitation and stirred for 3 hours to allow microsphere hardening and methylene chloride evaporation. Microspheres were collected by filtration and lyophilized for 24-48 hours for further methylene chloride evaporation. Microspheres were stored at -20° C until use.

5.2 The resulting EYE-001 aptamer containing microspheres were packed into a polypropylene chamber. Cyanoacrylate glue was placed on the border of the chamber and the chamber was adhered onto the left eyes (OS) of dutch-belted rabbits at a location about 5 mm away from the limbus of each eye. The PLGA of the packed microspheres, when in fluid communication with the highly hydrated sclera, degraded to release the nucleic acid aptamer from the microspheres. The released nucleic acid aptamer could then contact and pass through the sclera. The right eyes of each rabbit (OD) were used as

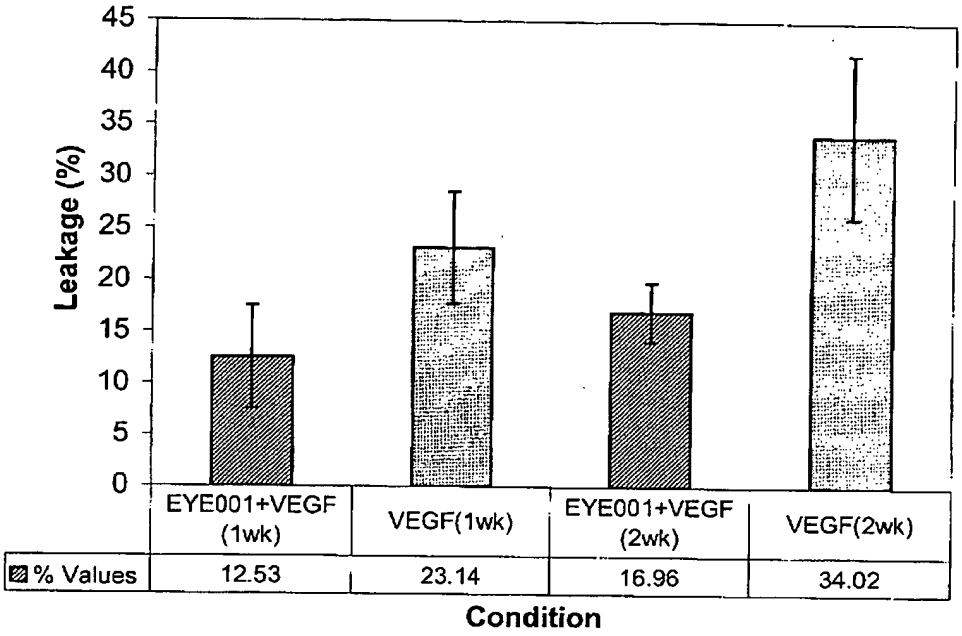
a control (i.e., no EYE-001 aptamer). The devices, once attached to each left eye, were left in place for one or two weeks.

5.3 The day before analysis, each eye received an intravitreal injection of 1 μg/mL of VEGF (R&D Systems) to trigger vascular permeability of the blood vessels within the eye. On the day of analysis, the rabbit femoral vein was cannulated with a 24 gauge catheter and Evans Blue dye was infused into the bloodstream over 10 seconds at a dosage of 45mg/kg. After 4 hours circulation time, the chest cavity was opened and the animals were perfused through the left ventricle at 37°C with 400 mL of citrate buffer (0.05M, pH 3.5) and subsequently with 500 mL of citrate-buffered paraformaldehyde (1% wt/vol, pH 3.5, Sigma). Immediately after perfusion (physiological pressure of 120 mm Hg), both eyes were enucleated and bisected at the equator. The retinas then were carefully dissected away under the operating microscope and thoroughly dried in a Speed-Vac for 4 hours. After measurement of the retinal dry weight, the Evans Blue dye was extracted by incubating each retina in 200 μL of formamide (Sigma) for 18 hours at 70°C. The extract was ultra-centrifuged (IEC Micromax RF) through Ultra free-MC tubes (30,000 NMWL Filter Unit, Millipore) at a speed of 6,000 rpm for 2 hours at 4°C. At exactly 2 hours after infusion of Evans Blue dye, immediately before the beginning of the perfusion, 1 mL of blood was drawn from the left ventricle to obtain the final concentration of Evans Blue dye. Sixty μL of the tissue-extracted Evans Blue dye supernatant and plasma-collected Evans Blue dye were used for triplicate spectrophotometric measurements. A background-subtracted absorbance was determined by measuring each sample at both 620 nm (the absorbance maximum for Evans Blue dye) and 740 nm (the absorbance minimum for Evans Blue dye). The concentration of the dye in the extracts was calculated from a standard curve of Evans Blue dye in formamide.

6. Results and Discussion

6.1 The results are summarized in Figure 1, where the values presented are the average result of 6 different experiments (n=6). The standard error = $\sigma/\sqrt{n}$ where σ is the standard deviation of the original distribution and $\sqrt{n}$ is the square root of the sample size.

Figure 1



6.2 As shown in Figure 1, the % leakage of Evans Blue dye in the control eyes after one and two weeks was 23% and 34%, respectively. However, when the EYE-001 aptamer was administered transclerally, the % leakage of Evans Blue dye after one and two weeks was reduced to 12.5% and 17%, respectively. At both the one and two week time points, the transcleral delivery of the EYE-001 aptamer reduced blood vessel leakage by about 50%. The results demonstrate that the EYE-001 aptamer, when delivered transclerally, can exert a biological effect *in vivo* by reducing, for example, the leakage from blood vessels within the eye.

6.3 In this experiment, the aptamer was encapsulated into PLGA microspheres merely to provide sustained delivery of the EYE-001 aptamer to the surface of the eye. I do not believe that encapsulation was necessary to facilitate delivery of the nucleic acid through the sclera.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: August 13, 2004

Karen. G. Carrasquillo, Ph.D.

3105880

Controlled Delivery of the Anti-VEGF Aptamer EYE001 with Poly(lactic-*co*-glycolic)Acid Microspheres

Karen G. Carrasquillo,[1] *Joseph A. Ricker,*[2] *Ioannis K. Rigas,*[1] *Joan W. Miller,*[1] *Evangelos S. Gragoudas,*[1] *and Anthony P. Adamis*[1]

PURPOSE. To develop a controlled-drug delivery system for the long-term inhibition of vascular endothelial growth factor (VEGF) and its mediated responses.

METHODS. Poly(lactic-*co*-glycolic)acid (PLGA) microspheres containing anti-VEGF RNA aptamer (EYE001) formulations in the solid-state were developed by an oil-in-oil solvent evaporation process. In vitro experiments were performed to characterize the release profiles. Stability and bioactivity of the released drug were assayed by monitoring the RNA aptamer's ability to inhibit VEGF-induced cell proliferation in human umbilical vein endothelial cells (HUVECs). Cell proliferation experiments were conducted with aptamer aliquots collected after short-, mid-, and long-term release time points. To investigate the feasibility of this polymer device as a potential transscleral delivery device, an in vitro apparatus was developed to assess polymer hydration and degradation through rabbit sclera and subsequent delivery through it.

RESULTS. PLGA microspheres were able to deliver EYE001 in a sustained manner, with an average rate of 2 μg/d over a period of 20 days. Solid-state stabilization of the aptamer with disaccharide trehalose before lyophilization and encapsulation in PLGA rendered the drug more stable after release. Cell proliferation experiments demonstrated that the bioactivity of the aptamer was preserved after release, as indicated by inhibition of endothelial cell proliferation after incubation with VEGF. Microspheres packed into a sealed chamber and placed onto the "orbital" part of a rabbit sclera for a period of 6 days became hydrated and started to degrade, as shown by scanning electron microscopy (SEM). As a result, the aptamer was delivered from the microspheres through the sclera, as determined spectrophotometrically.

CONCLUSIONS. The loading of aptamer-containing microspheres into a device and placing it on the orbital surface of the sclera was assessed and shown to be feasible. RNA aptamer EYE001 encapsulated in PLGA was delivered over a period of 20 days with retained activity. This method represents a promising approach for the transscleral delivery of drugs and the treatment of choroidal and retinal diseases. (*Invest Ophthalmol Vis Sci.* 2003;44:290-299) DOI:10.1167/iovs.01-1156

Vascular endothelial growth factor (VEGF) has been identified as a key positive regulator of angiogenesis.[1] It acts as an endothelial cell mitogen and chemoattractant in vitro[2,3] and induces vascular permeability and angiogenesis in vivo.[2,3] Elevated VEGF expression is correlated with several forms of ocular neovascularization that often lead to severe vision loss, including diabetic retinopathy,[4] retinopathy of prematurity,[5] and macular degeneration.[6] Thus, agents that specifically inhibit VEGF may have great utility in combating a variety of human diseases for which few effective treatments are presently available.[7]

Recently, a method used to isolate oligonucleotide ligands (aptamers) from libraries of RNA, DNA, or modified nucleic acids that bind with high affinity and specificity to various molecular targets, including proteins and peptides, has been described.[7] In particular, an RNA-based aptamer has been developed with high affinity toward $VEGF_{165}$.[8] After being isolated and determined to bind specifically to $VEGF_{165}$, RNA aptamer EYE001 (formerly referred to as NX1838) was further modified chemically to render it nuclease-resistant and thermally more stable, thus enhancing its potential for therapeutic utility.[7,8] The promising results displayed by the aptamer's biological response both in vitro and in vivo against diseases associated with the growth of new blood vessels or angiogenesis, especially those threatening to vision, suggest that it has excellent potential as a therapeutic agent.[7-9] Currently, EYE001 is undergoing clinical trials for the treatment of age-related macular degeneration (AMD). Because choroidal neovascularization is a severe complication of AMD and because patients with subretinal neovascularization, including those with AMD, show increased expression of VEGF, it is believed that antiangiogenic and/or antivascular permeability factors could delay or reverse the pathogenesis of AMD.[10-12]

Widespread clinical use of EYE001 will necessitate a practical and effective method of delivery to the eye. Application in current clinical trials relies on intravitreal injections of the aptamer. Although this method allows for assessment of the potential use of the aptamer as a therapeutic drug, it is a less than optimal way to treat patients on a day-to-day basis, because of its invasive nature. The mode of delivery should provide exposure to the drug for the required period it must be minimally invasive and, preferably, localized.

Recent studies have highlighted the applicability of transscleral delivery for various macromolecules, including globular proteins.[13,14] The potential for transport or diffusion through the sclera lies in the large and accessible surface area of this tissue, its high degree of hydration, hypocellularity, and permeability that does not decline significantly with age.[15-17] Thus, this approach would circumvent the limitations and problems presented by other modes of delivery to treat posterior segment diseases—intravitreal, systemic, and eye drops—that include, among other drawbacks, retinal detachment, systemic side effects, and diffusional limitations, respectively.[18-20]

From the [1]Angiogenesis and Retina Research Laboratory, Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts; and the [2]Electron Microscopy Laboratory, Draper Laboratories, Cambridge, Massachusetts.

Supported by Eyetech Pharmaceuticals (APA), the Roberta W. Siegel Fund (APA), National Eye Institute Grants EY12611 and EY11627 (APA), the Juvenile Diabetes Foundation (APA), the Falk Foundation (APA), and the Iaccoca Foundation (APA).

Submitted for publication November 19, 2001; revised May 17, 2002; accepted June 18, 2002.

Commercial relationships policy: F (KGC, IKR, APA); P (JWM, ESG, APA); C (APA); N (JAR).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Anthony P. Adamis, Massachusetts Eye and Ear Infirmary, 325 Cambridge Street, Boston, MA 02114; tony_adamis@meei.harvard.edu.

Because certain methods of transscleral delivery can be destructive (i.e., iontophoresis), causing, in some cases, retinal necrosis and gliosis,[21] we have focused our attention on biodegradable polymer sustained-delivery devices. Sustained delivery of proteins or nucleic acids from polymer matrixes offers the advantage of targeting specific tissues and increasing the comfort and compliance of patients.[22-24] Specifically, we have used poly(lactic-*co*-glycolic) acid (PLGA) as the encapsulation matrix of choice. PLGA, an FDA-approved material, has been extensively studied for its biocompatibility, toxicology, and degradation kinetics.[25,26] It has been used clinically as a suture material since the 1970s,[27] and recently it has been used as scaffold in tissue engineering techniques.[28,29] An important characteristic of PLGA carrier systems is their ability to be applied locally, which allows intralesional concentrations of the drug to be sustained while systemic deleterious side effects are minimized, thus providing a pharmacological advantage at the treatment site.[30,31] In vivo studies in which PLGA was used as a carrier system to the eye for various types of drugs used to treat various diseases have reported no sign of ocular toxicity or significant inflammatory responses for periods of up to 2 months.[31,32] These studies, however, involved either the encapsulation of small synthetic drugs and molecules or the intravitreal injection of such polymer devices after a sclerotomy, which in itself is invasive.

In our present study, we considered the potential of transscleral delivery of drugs in a sustained and controlled manner in an in vitro setup. We were able to deliver the anti-VEGF aptamer EYE001 for a period of up to 20 days in a biologically active state, showing no destabilization due to the encapsulation procedure. We hypothesize that delivering drugs in a sustained manner through the sclera is a viable approach for the treatment of various vision-threatening diseases.

Methods

Lyophilized RNA Aptamer EYE001

EYE001 was produced at Gilead Sciences, Inc. (Boulder, CO) by the systematic evolution of ligands by exponential enrichment (SELEX) process as described[7,33] and supplied as a liquid formulation of 3 mg/200 μL saline solution. EYE001 is a pegylated RNA aptamer of 50 kDa, with an A-type secondary structure, 40 mg/mL solubility, and a net negative charge of -28. Samples were lyophilized in a (model SNL315SV; Savant Instruments, Farmingdale, NY) at a chamber pressure of 80 mbar and a shelf temperature of - 45°C for 48 hours to obtain excipient-free aptamer. The lyophilized material was sealed in sterilized glass vials and stored at - 20°C until use. Lyophilized samples containing trehalose (Sigma Chemical Co., St. Louis, MO) at a 1:3 weight ratio were prepared by adding an appropriate amount of concentrated excipient solution to the excipient-free aptamer solution before lyophilization.[34,35] The ratio was selected based on the mass amounts of aptamer to trehalose needed to stabilize aptamer structure and function on lyophilization and thus prevent lyophilization-induced structural changes.

Circular Dichroism Experiments

Circular dichroism (CD) spectra were recorded on a CD spectrometer (model 202; Aviv Instruments, Lakewood, NJ). Data were collected at 25°C using a bandwidth of 0.5 nm and an average time of 0.1 second. The CD spectra were collected from 200 to 330 nm with a 0.5-cm quartz cells and corrected for the phosphate buffer signal contribution measured under identical conditions.

Microsphere Preparation

PLGA microspheres were prepared by a non-aqueous oil-in-oil methodology, as described previously.[24] Briefly, 25 to 30 mg of solid aptamer was suspended in a solution of 200 mg/2 mL PLGA (Resomer 502 H, i.v. (inherent viscosity) 0.16-0.24 dL/g, 0.1% in chloroform, 25°C, molecular weight [M_w] 10 to 12 kDa, half-life for degradation approximately 1 to 1.5 months; Boehringer Ingelheim Pharma KG, Ingelheim, Germany) in methylene chloride with a homogenizer (Polytron, model PT 1200C; Brinkman, Westbury, NY) using a standard 12-mm diameter generator at approximately 20,000 rpm for 1 minute. After suspension of the aptamer, the coacervating agent poly(dimethylsiloxane) was added at a rate of 2 mL/min under constant homogenization, to ensure homogeneous dispersion of the coacervating agent, phase separation of PLGA dissolved in methylene chloride, and formation of microspheres. The coacervating mixture containing the microspheres was then poured into an Erlenmeyer flask containing 50 mL heptane under constant agitation and stirred for 3 hours at room temperature to allow for hardening of the microspheres. Microspheres were collected by filtration with the use of a 0.22-μm nylon filter, washed twice with heptane, and dried for 24 hours at a vacuum of 80 mbar.

Encapsulation Efficiency

Encapsulation efficiency was determined as described previously.[23] Ten milligrams of PLGA microspheres was placed in 2 mL methylene chloride and stirred for 30 minutes to dissolve the polymer. The solution was then centrifuged at 10,000 rpm for 10 minutes to precipitate the insoluble RNA aptamer, supernatant was removed, and the remaining methylene chloride was allowed to evaporate. To ensure evaporation of the methylene chloride, the sample was placed in a vacuum for 24 hours. The aptamer was then dissolved in Dulbecco's phosphate-buffered saline (DPBS; GibcoBRL, Grand Island, NY), and the concentration of entrapped aptamer in PLGA was determined spectrophotometrically. The percentage encapsulation efficiency was calculated by relating the experimental aptamer entrapment to the theoretical aptamer entrapment: (experimental/theoretical) × 100.

In Vitro Release Profiles

Ten milligrams of solid microspheres was placed in 2 mL of DPBS, 1× (pH 7.3) and incubated at 37°C. Every 24 hours, the microspheres were gently centrifuged at 500 rpm for 1 minute, and the supernatant was removed for determination of aptamer concentration at 260 nm, ε_{PBS} = 25.08 cm^{-1} $(mg/mL)^{-1}$ as described.[24,36] Microspheres were then resuspended in 2 mL fresh DPBS to maintain sink conditions and control the pH.[24,37] Ten milligrams of blank (empty) PLGA microspheres were subjected to the same conditions as PLGA-loaded microspheres, and the supernatant collected from these was used as a blank in the spectrophotometric analysis. Data are presented as the average of three independent experiments with standard deviations.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were obtained from Cascade Biologics, Inc. (Portland, OR). Cells were maintained in growth-factor supplemented medium, including 2% vol/vol fetal bovine serum (FBS), 1 μg/mL hydrocortisone, 10 ng/mL human epidermal growth factor, 3 ng/mL basic fibroblast growth factor, and 10 μg/mL heparin under standard tissue culture conditions (5% CO_2, 37°C, 100% relative humidity). Medium was changed every 48 to 72 hours, and cells were passaged by standard trypsinization and plated at a cell concentration of 2.5 × 10^3 cells/cm^2.

VEGF-Induced Endothelial Cell Proliferation

Proliferation assays were performed as described previously[7] with few variations. Briefly, HUVECs were seeded into 6-well or 12-well plates (2.5 × 10^3 cells/cm^2) as required in growth-factor-deficient medium (Medium 200; 5% FBS, 1 μg/mL heparin; Cascade Biologics) for 24 hours before experimentation. Aptamers (10 nM) collected after release from PLGA microspheres at specific time points and then

$VEGF_{165}$ (10 ng/mL; R&D Systems, Minneapolis, MN) was added to cells and incubated for 4 days. Cells were trypsinized and counted with a cell counter (model Z1; Coulter, Beds, UK). Wells containing cells without addition of aptamer or $VEGF_{165}$ were trypsinized and counted for basal growth estimation (blanks).

Scanning Electron Microscopy

Samples were affixed with double-sided carbon tape to an aluminum stub and sputtered with approximately 100 nm gold (Sputter Coating System; SPI, West Chester, PA). SEM images were then obtained (model S360; Cambridge Instruments, Monsey, NJ) .

Isolation and Preparation of Rabbit Sclera

The experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and guidelines developed by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. Dutch belted rabbits (Myrtle's Rabbitry, Inc., Thompson Station, TN), each weighing 2 to 3 kg, were anesthetized and killed with an intramuscular combination of 40 mg/kg ketamine (Abbott Laboratories, North Chicago, IL) and 10 mg/kg xylazine (Bayer, Shawnee Mission, KS) as described.[14] The eyes were enucleated immediately before the rabbits were killed and were immersed in DPBS. The adherent muscles were excised, and scleral tissue was removed. Areas free of nerve and vessel emissaries were used to obtain 12 × 20-mm slices of sclera under microscope caliper guidance. Each piece of sclera was immersed on PBS and used on the day of isolation.

In Vitro Transscleral Delivery Apparatus

The in vitro apparatus used for these experiments was modified from one previously described (Fig. 1).[14] Briefly, a 10 × 18-mm window was created on one face of a polystyrene cuvette (Sigma, St. Louis, MO) with use of a vertical milling machine (Bridgeport Machines; Bridgeport, CT), and a piece of sclera was blotted dry and placed over this window without stretching, avoiding asymmetrical stress. The tissue was sealed to the cuvette with a small amount of cyanoacrylate tissue adhesive (Ellman International, Hewlett, NY) applied continuously around its rim. PLGA-loaded solid microspheres (5 mg) were packed into a device 9 mm in diameter and 4 mm in depth made from a polypropylene cap of a 26.5-gauge needle (BD Biosciences, Lincoln Park, NJ). Cyanoacrylate tissue adhesive was placed around the border of the device and sealed against the orbital surface. A second identical cuvette was aligned with the first cuvette and glued in place along the margins of the tissue. Both sides of the cuvette were then filled with DPBS (2.5 mL), and the apparatus was placed in an incubator at 37°C without agitation. One side was considered the "uveal" chamber where diffusion of the aptamer would occur if the delivery were successful. The other side facing the "orbital" surface of the sclera would comprise any part of the sclera not covered by the device containing the microspheres and would serve as a control to assess any leakage from the device. A protease inhibitor cocktail (Complete Mini; Roche Diagnostics, Indianapolis, IN), at concentrations recommended by the manufacturer, was added to avoid proteolytic degradation of the tissue. In addition, 0.1 mM sodium azide was added to inhibit growth of bacteria in the medium.[17] After 24 hours the "uveal" chamber was sampled for aptamer concentration at 260 nm with a spectrophotometer (UV-Vis LambdaBio 40; Perkin Elmer, Wellesley, MA), and the "orbital" chamber was sampled as a control. Each side was replenished with fresh DPBS. To assess microsphere hydration and degradation, scleral tissue was analyzed by SEM after incubation for a determined period.

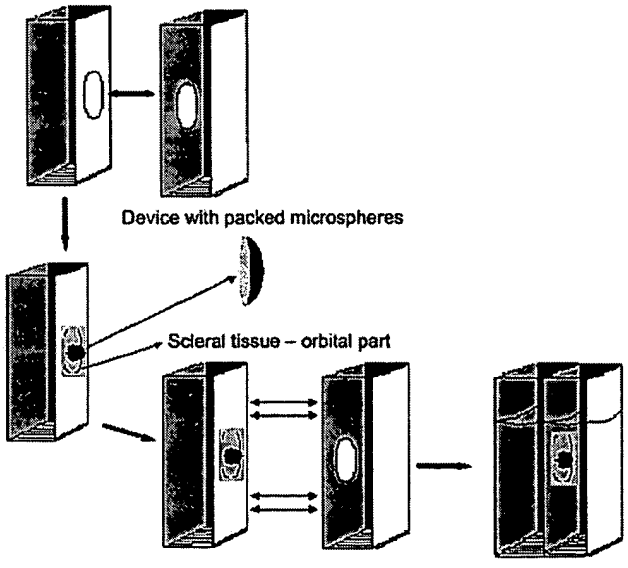


FIGURE 1. Schematic of the in vitro transscleral delivery apparatus.

Transmission Electron Microscopy

Tissue was placed in modified Karnovsky fixative consisting of 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer with 8 mM $CaCl_2$ and fixed for 12 to 24 hours at 4°C. The specimens were subsequently changed to 0.1 M cacodylate buffer for storage at 4°C. The tissue was trimmed to block size and postfixed in 2% aqueous OsO_4 for 2 hours at room temperature. After the tissue was rinsed in buffer, it was dehydrated in ascending concentrations of ethanol, transitioned through propylene oxide, and infiltrated with mixtures of propylene oxide and Epon (EMBed 812; Electron Microscopy Sciences, Fort Washington, PA), embedded in pure Epon, and polymerized at 60°C for 18 to 24 hours. One-micrometer sections and thin sections were cut on an ultramicrotome (Ultracut E; Leica, Deerfield, IL). The 1-μm sections were stained with 0.5% toluidine blue and the thin sections with saturated aqueous uranyl acetate and Sato lead stain, and then examined with a transmission electron microscope (model CM-10 Philips, Eindhoven, The Netherlands).

Statistics

The paired Student's two-tailed t-test was used to compare cell counts after each incubation condition. An α level of 0.05 was used as the criterion to reject the null hypothesis of equality of means.

RESULTS

Characteristics of PLGA Microspheres

SEM images of the microspheres after preparation indicated the formation of nonporous spheres with an average diameter of 14 ± 4 (Fig. 2A) and 16 ± 4 μm (Fig. 2B) after hydration. The encapsulation efficiencies of aptamer into PLGA varied with the original amount of drug used as starting material. Encapsulation efficiency for microspheres containing aptamer colyophilized with trehalose was 80% ± 5% when 32.1 mg was used as the starting material, whereas for excipient-free aptamer-containing microspheres, the encapsulation efficiency was 71% ± 2% when 6.6 mg was used. Analysis of the microspheres after 10 days of release showed degradation of the polymer matrix and the formation of pores through which the aptamer was slowly released (Fig. 2B).

RNA Aptamer EYE001 Release from PLGA Microspheres

In vitro release profiles (Fig. 3A) for both excipient-free aptamer and aptamer colyophilized with trehalose at a 1:3 weight ratio of aptamer to trehalose (herein referred to as

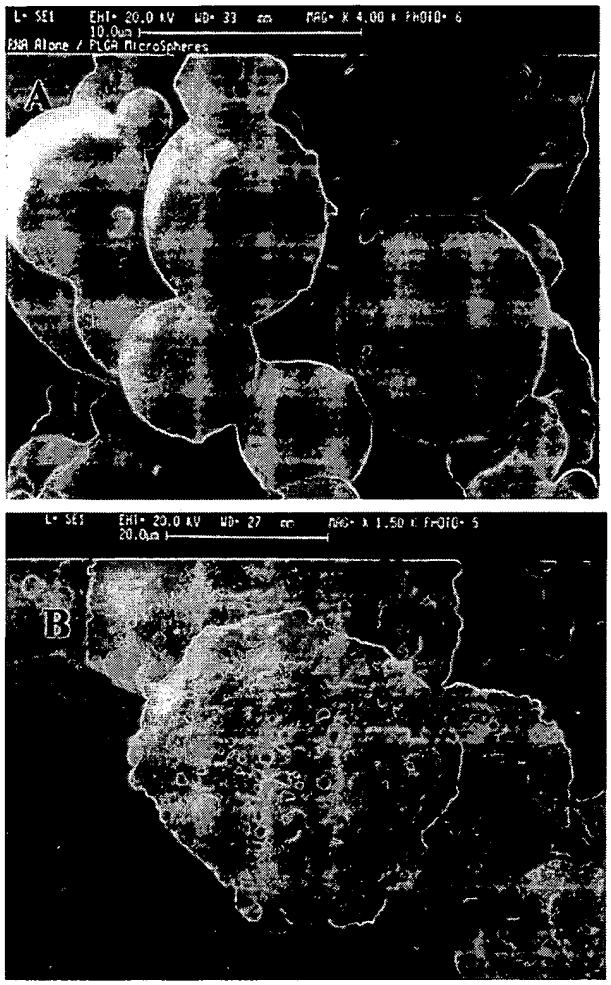


FIGURE 2. (A) PLGA microspheres loaded with anti-VEGF aptamer EYE001 before incubation with release medium and (B) after 10 days of exposure to aqueous release medium. The average diameter of the microspheres was 14 ± 6 μm. Determination of average diameter was a result of diameter estimations of approximately 30 individual microspheres.

EYE001-Tre) exhibited a controlled release of the drug in a period of more than 20 days. Release kinetics were characterized by a very low-burst release during the first 24-hour period, followed by a continuous release with no evidence of a lag phase. Both formulations were completely released, indicating no adsorption of the aptamer to the polymer core. The average amount of drug released was 2 μg/d, regardless of the amount of drug originally encapsulated. The encapsulation efficiency for both formulations in PLGA was 70% to 85%, with a theoretical loading of 3.95% and actual loading of 2.76% ± 0.80%, indicating that the presence of trehalose had no effect in the encapsulation efficiency of the polymeric system.

Secondary Structural Determination of EYE001 Formulations upon Lyophilization

To assess any structural changes due to the nature of the formulation of EYE001 upon lyophilization, EYE001 formulations lyophilized as described herein were reconstituted in PBS and its CD spectra determined and compared with an aqueous EYE001 standard. Given that EYE001 has an A-type RNA structure (duplex formation, right-handed helix),[7] the CD spectra exhibit a maximum of approximately 260 nm and a minimum of approximately 210 nm.[38,39] A decrease in molar ellipticity in either maxima or minima is a reflection of a secondary structural change.[38,39] The CD spectrum of EYE001 in the absence of any excipient on lyophilization and further reconstitution exhibited a slight decrease in intensity at both wavelengths. It was observed that when increasing the mass ratio of the disaccharide stabilizer trehalose[22-24,34,35] to EYE001 before lyophilization, there was an improvement in the retention of structure, as evidenced by molar ellipticities at both wavelengths comparable with those of the aqueous EYE001 standard (Fig. 4).

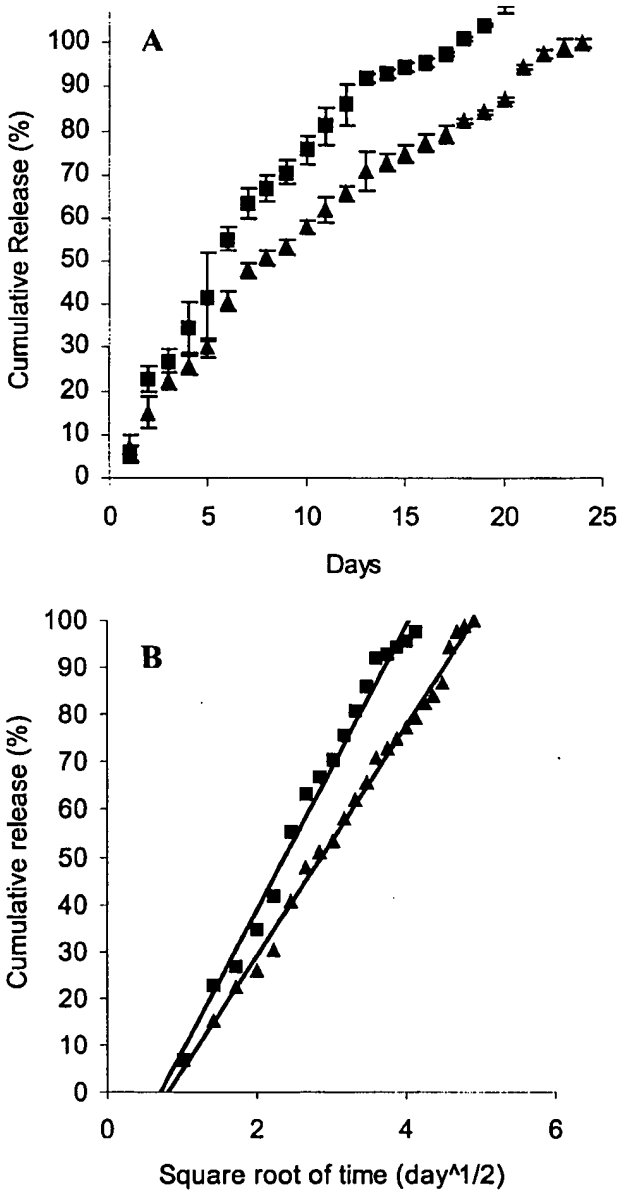


FIGURE 3. (A) Cumulative release profile of (■) excipient-free EYE001 and (▲) EYE001-Tre from PLGA microspheres. (B) Correlation between the amount of EYE001 released and the square root of time for (■) excipient-free EYE001 and (▲) EYE001-Tre. The correlation coefficients of the released formulations were 0.9852 and 0.9946, respectively.

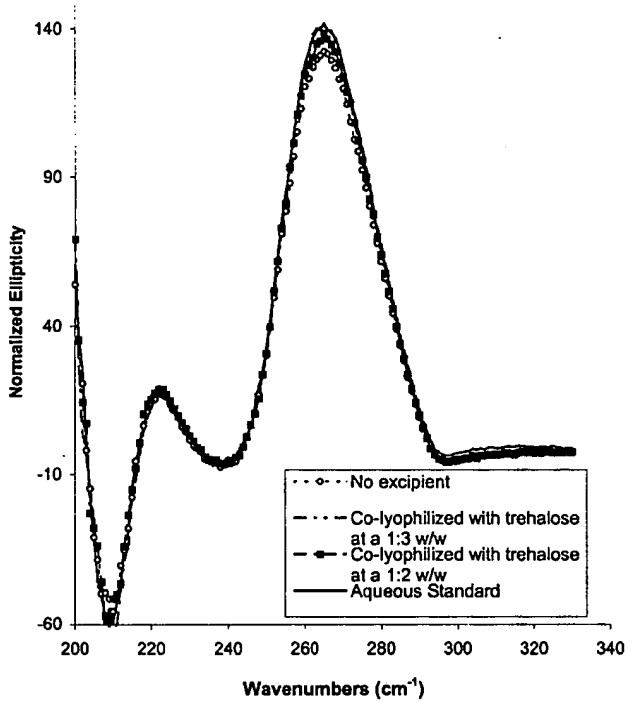


FIGURE 4. CD spectra of reconstituted EYE001 formulations under different lyophilization conditions. The spectra were recorded after reconstitution of samples in PBS.

Anti-VEGF Aptamer Activity after Release from PLGA

EYE001 activity after encapsulation and further release from PLGA microspheres was assayed by monitoring its ability to inhibit VEGF-induced proliferation of HUVECs. To determine the feasibility of polymer microspheres as a viable delivery device, proliferation assays were conducted at various stages during the release period. The representative time points chosen were at early, intermediate, and late stages of release. We were interested not only in the stability of the aptamer over time but also in the stability of the formulation state in which the aptamer was encapsulated in PLGA.

The VEGF-induced proliferation of HUVECs after a period of 4 days showed a threefold average increase in cell counts compared with those found in blanks. On HUVEC incubation with VEGF in the presence of the different aptamer formulations after release, it was evident that regardless of the formulation state, the aptamer was capable of at least partially inhibiting VEGF-induced cell proliferation (Fig. 5). However, it is worth noting that the inhibition showed by the aptamer was, in general, enhanced when EYE001 was colyophilized in the presence of trehalose and then encapsulated in PLGA. EYE001 preserved its bioactivity after encapsulation in PLGA and during its release over a period of 20 days (Fig. 5). Incubation of HUVECs with PLGA-loaded microspheres containing EYE001 formulations and degraded PLGA supernatant had no effect on HUVEC proliferation (data not shown).

Transscleral Delivery of EYE001 Released from PLGA Microspheres

After active EYE001 was delivered from PLGA microspheres in a controlled manner for an extended period in vitro, we sought to determine whether the hydration of the sclera would be sufficient to degrade the microspheres and result in aptamer release and diffusion through the sclera. To do this, PLGA-loaded microspheres were loaded into a device and placed it on the sclera of Dutch belted rabbits as described in Figure 1 (see the Materials and Methods section). The degree of polymer degradation was monitored qualitatively by analyzing the morphology of the microspheres. SEM pictures show the morphologic state of the microparticles after exposure to scleral hydration after a period of 18 hours and after 6 days (Fig. 6). During the first 18 hours, the polymer microspheres seemed to adhere to the tissue, but no significant degradation was observed (Fig. 6B), as expected, because of the short incubation time. However, after 6 days, PLGA microspheres showed significant degradation and formation of pores along its surface (Fig. 6C). The visible signs of degradation indicated that scleral hydration was sufficient to degrade the PLGA-loaded microspheres, indicating feasibility of the delivery method for EYE001 through the sclera.

To determine whether diffusion of EYE001 through the sclera was indeed possible after delivery from PLGA microspheres, aptamer concentration was monitored in the uveal chamber (sampling the chamber with the uveal side of the sclera exposed), and, as a control, the aptamer concentration in the orbital chamber was monitored as well (sampling chamber with the orbital side of sclera exposed and containing the device loaded with microspheres). Having determined the characteristics of the in vitro release profiles of EYE001 from the microspheres, aptamer diffusion through the sclera was monitored for 6 days. Table 1 presents the data showing the amount of aptamer diffused through the sclera. As can be observed, the amount of aptamer delivered from PLGA microspheres and diffused through the sclera is comparable with that released in vitro from isolated microspheres. An average of 2 μg/d was sampled in the uveal chamber, indicating that EYE001 diffused readily through the sclera, as reported previously for molecules of similar molecular weight.[14] An average of 0.5 μg/d was sampled in the control chamber. SEM analysis of lyophilized powder obtained after freeze drying of the volume sampled in the uveal chamber revealed that there were no microspheres present, indicating that the drug permeated in its free, nonencapsulated form.

Given that we monitored diffusion for 6 days in an in vitro setup, an important consideration was the integrity and viability of the sclera during the transport study. To this end, we examined cultured scleral tissue immersed in PBS and incubated at 37°C for 6 days by transmission electron microscopy (TEM). As a control, we analyzed a fresh scleral tissue, fixed the same day it was detached. As can be observed in Figure 7, there were signs of swelling of the collagen fibrils in the cultured sclera when compared with fresh rabbit sclera, as evidenced by the thickness of the collagen fibers, but the general ultrastructure of the tissue was preserved, as determined by TEM. This is consistent with the observations in other investigations in which similar in vitro experiments were performed to determine diffusion of solutes through the sclera, with the results indicating that normal scleral physiology can be maintained over the course of short- and long-term perfusion periods.[40]

DISCUSSION

The goal of the present study was to develop a drug delivery modality that could release the anti-VEGF aptamer EYE001 in a sustained and controlled manner over a significant period and could be applied locally to the outer part of the sclera. The retina and choroid are the target tissues, because this aptamer is intended to block the contribution of VEGF to choroidal neovascularization and diabetic macular edema, respectively. Transscleral administration, no more frequently than every 6

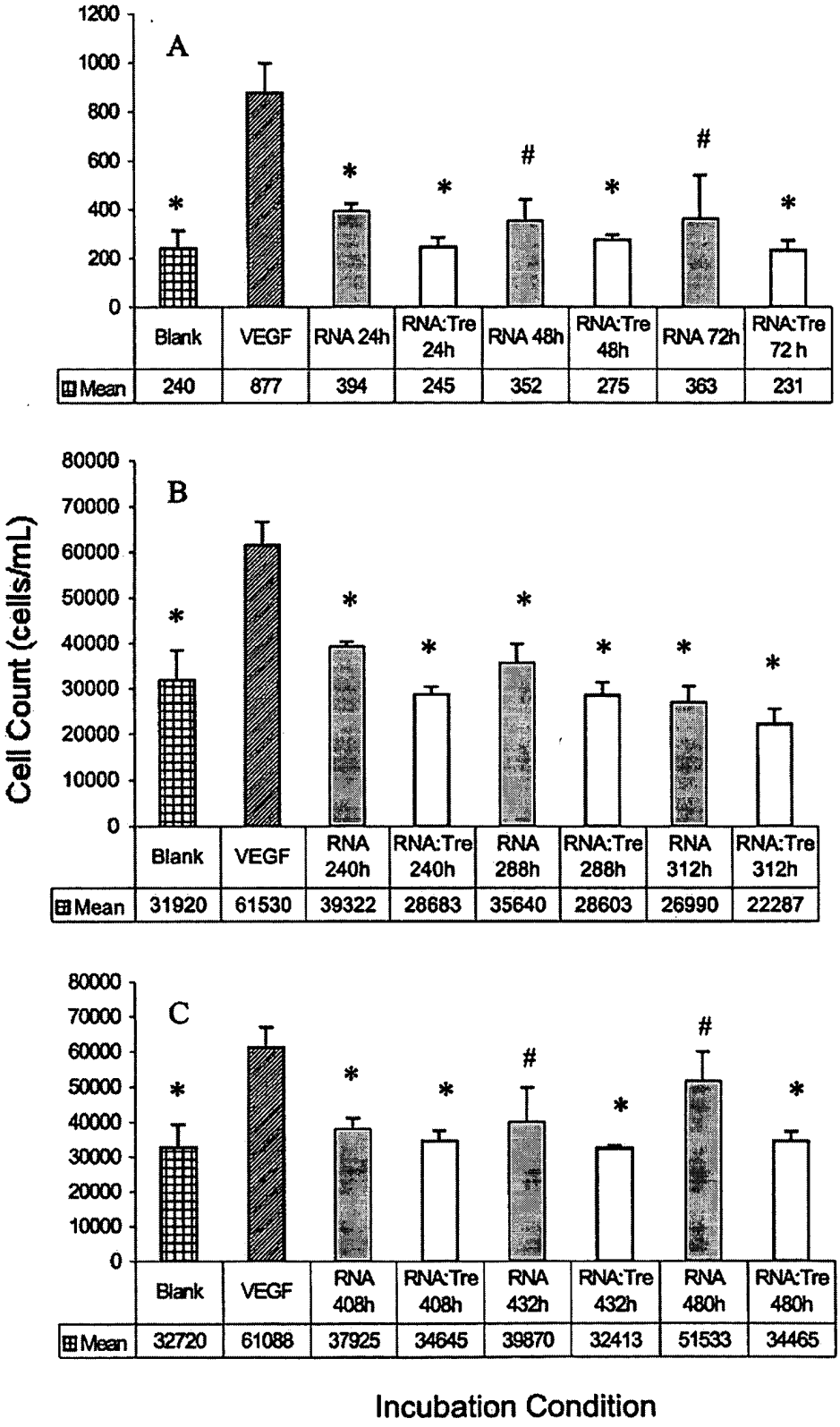


FIGURE 5. Cell proliferation assays of HUVECs incubated with EYE001 formulations after release from PLGA microspheres. Each graph indicates the incubation condition and the time-point at which EYE001 was collected after release from PLGA. The mean values represent the average cell count results for each condition in three independent experiments. (A) Short-, (B) mid-, and (C) long-term release time points. $*P < 0.05$; $\#P > 0.05$ versus VEGF-induced cell proliferation. $n = 3$ for all time points.

weeks, would prove an attractive substitute to intravitreal injections of the aptamer, currently occurring at a similar frequency in two separate clinical trials.

For this purpose, the biodegradable, biocompatible, and FDA-approved polymeric material PLGA was selected.[23] The release profiles of EYE001 from these microspheres were characterized by a low initial burst, followed by continuous release in the absence of a lag phase. Typical release profiles from PLGA microspheres are triphasic, characterized by an initial burst as drug entrapped near the surface releases, followed by

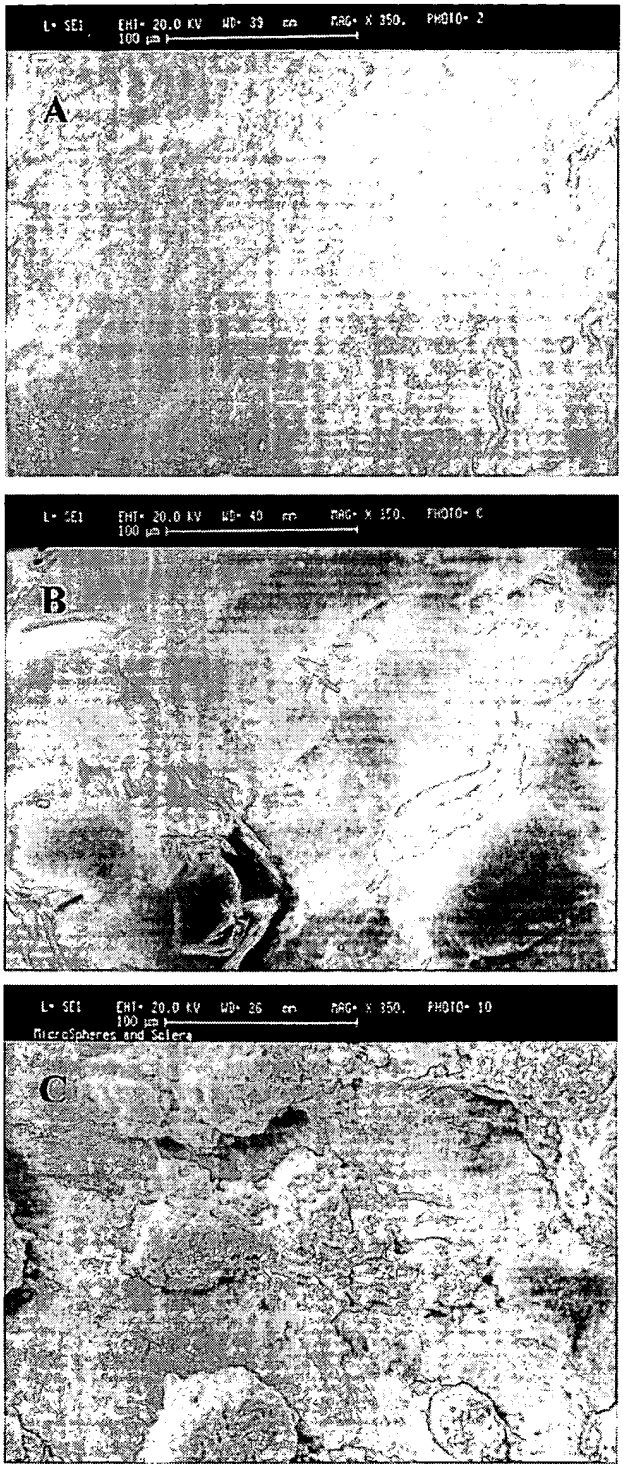


FIGURE 6. SEM images of (A) bare rabbit sclera and (B) rabbit sclera with PLGA microspheres after 18 hours and (C) 6 days of exposure to the orbital surface.

a lag phase controlled by polymer degradation and final release of the drug as it diffuses from the polymer core as erosion takes place.[26] In the scenario observed, it is probable that EYE001 formulations encapsulated in PLGA were homogeneously distributed throughout the polymeric matrix. As described by Higuchi[41,42] and reported elsewhere,[43,44] when a drug releases from a homogeneous matrix-type delivery system, the process is diffusion-controlled and is evidenced by a proportionality between the amount of drug being released and the square root of time. The process is described by the following equation:

$$Q = \sqrt{2WDC_s t}$$

where Q is the rate of released drug, D is the diffusion coefficient of the drug in the matrix, W is the total amount of the drug per unit volume of matrix, C_s is the solubility of the drug in the matrix, and t is the drug release time.

The release of both excipient-free aptamer and EYE001-Tre from PLGA as a function of the square root of time ($t^{1/2}$) show a linear relationship with correlation coefficients of 0.98 and 0.99, respectively (Fig. 3B). These data support the hypothesis that both aptamer formulations were released through a diffusion-controlled process.

An important consideration in our development of a long-term delivery device for a nucleic acid such as EYE001 was its stability before, during, and after the encapsulation process in PLGA. Nucleic acids are known to suffer depurination and become susceptible to free radical oxidation in aqueous solutions.[45,46] Such a phenomenon was recently reported by a group evaluating the potential development of pharmaceutical formulations of plasmid DNA with long-term storage stability.[47] To this end, we colyophilized EYE001 with the known potent stabilizer trehalose,[34,48] and used a completely nonaqueous oil-in-oil methodology[48,49] for the creation of polymer microspheres that has been effective in the delivery of biologically active proteins with native secondary structures.[24,48-51]

The cell proliferation assays conducted to monitor aptamer bioactivity after release from PLGA microspheres reveal that the conditions chosen to create the polymer microspheres were satisfactory. As shown in Figure 5, EYE001 preserved its ability to inhibit VEGF-induced cell proliferation during all the representative time points along its release from PLGA. Although bioactivity was retained regardless of its formulation state, an improved level of bioactivity was observed in general when EYE001 was colyophilized with trehalose before encapsulation.

Incubation of PLGA microspheres directly with HUVECs revealed the same trend as that of the aptamer collected after it was released from isolated microspheres in vitro. No evident signs of toxicity or cell death were observed when blank PLGA microspheres were incubated with HUVECs from microscopic observations and cell counts (data not shown). These results are in agreement with reports by other groups that conducted cell proliferation assays with polylactides of various molecular weights with rat epithelial cells, human fibroblasts, and osteosarcoma cells under culture conditions.[52] Overall, it was determined that satisfactory biocompatibility was exhibited.[52,53]

TABLE 1. Amount of Aptamer Diffused through the Sclera after Release from PLGA Microspheres

Day	$EYE001_{SC}$* (μg)	$EYE001_{CC}$† (μg)
1	3.4 ± 0.8	0.7 ± 0.3
2	2.3 ± 0.5	0.5 ± 0.3
3	2.1 ± 0.6	0.5 ± 0.2
4	1.8 ± 0.3	0.6 ± 0.4
5	2.4 ± 0.1	0.5 ± 0.2
6	2.6 ± 0.2	0.4 ± 0.3

Data are presented as the average results of three experiments ± SD.

* Sampling Chamber.

† Control Chamber.

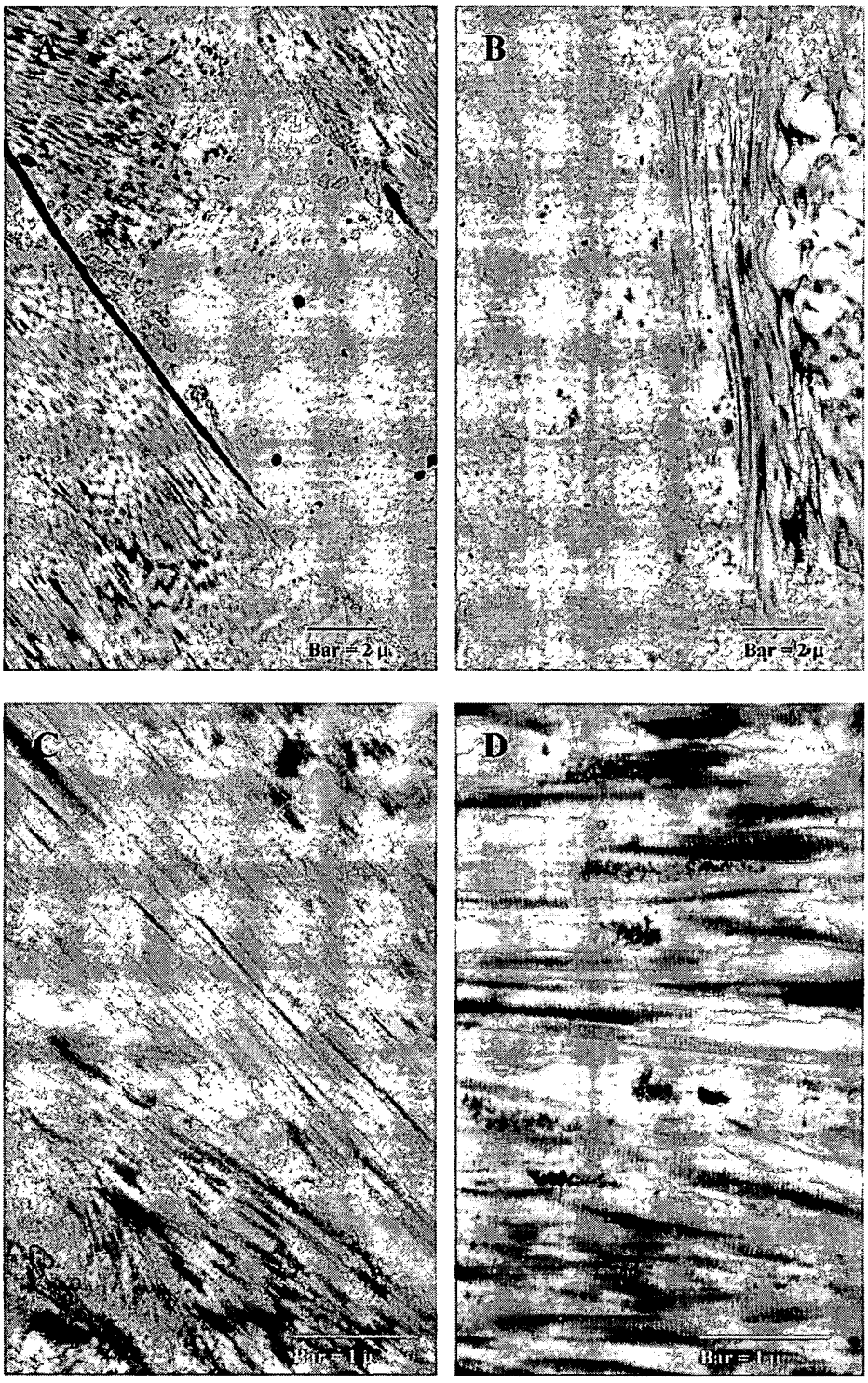


FIGURE 7. Cross-sections (A, B) and longitudinal sections (C, D) of rabbit sclera analyzed by TEM. Fresh rabbit sclera (A, C) was used as control to compare with sclera incubated in PBS at 37°C for 6 days (B, D).

These data add support for the conclusion that the method described in this report holds promise for the long-term inhibition of VEGF-mediated responses in vivo.

Rabbit sclera is 71% water[17] and, as documented by electron microscopy (Fig. 6C), it served to hydrate and degrade the solid PLGA microspheres placed on the orbital side of the sclera, which were not in contact with any hydration medium other than the hydrated scleral surface itself. An important aspect of PLGA controlled-delivery devices is that they provide continuous release and avoid the repeated use of injections or high concentrations of drug to achieve the desired pharmacological response. Even though controversy exists over how the flux over the sclera occurs and whether it achieves steady state,[54] our controlled-drug delivery device would increase drug-sclera contact, thus improving scleral absorption. The hypocellularity[55] and large surface area[15] of the human sclera, as well as its remarkable tolerance of foreign bodies overlying its surface (e.g., scleral buckles[56]) helps to facilitate diffusion through it and allow a long-term transscleral delivery device to be clinically feasible.

In this report, we present data showing the feasibility of delivering the anti-VEGF aptamer EYE001 in a sustained and controlled manner and in a biologically active form. The development of such an approach to drug delivery accompanies the advent of many potential antiangiogenic drugs for the treatment of various vision-threatening diseases that affect the posterior segment of the eye. Validation of this study would require testing the system in an in vivo model that would also address other important questions: how choroidal blood flow affects transscleral delivery and whether the concentrations of active drug delivered through the proposed system are sufficient to inhibit some or all the responses triggered by neovascularization in the posterior segment, among others. These studies are currently in progress in our laboratory.

Acknowledgments

The authors thank Norman Michaud, Director of Morphology, from the Howe Laboratory, Division of Ophthalmology of the Massachusetts Eye and Ear Infirmary for assistance with the transmission electron microscopic studies, Jeffrey T. Borenstein, Draper Laboratories, for assistance with the scanning electron microscopy, and Carlos J. Bosques, Department of Chemistry, Massachusetts Institute of Technology, for assistance with the circular dichroism studies.

References

1. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med.* 1995;1:27-31.
2. Leung D, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science.* 1989;246:1306-1309.
3. Keck P, Hauser SD, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science.* 1989;246:1309-1312.
4. Aiello L, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *New Engl J Med.* 1994;331:1480-1487.
5. Pierce EA, Avery RL, Foley ED, et al. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. *Proc Natl Acad Sci USA.* 1995;92:905-909.
6. Kvanta A, Algvere PV, Berglin L, et al. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. *Invest Ophthalmol Vis Sci.* 1996;37:1929-1934.
7. Ruckman J, Green LS, Beeson J, et al. 2'-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. *J Biol Chem.* 1998;273:20556-20567.
8. Bell C, Lynam E, Landfair DJ, et al. Oligonucleotide NX1838 inhibits VEGF165-mediated cellular responses *in-vitro*. *In Vitro Cell Dev Biol Anim.* 1999;35:533-542.
9. Soffer S, Moore JT, Kim E, et al. Combination antiangiogenic therapy: increased efficacy in a murine model of Wilms tumor. *J Pediatr Surg.* 2001;36:1177-1181.
10. Asayama N, Shimada H, Yuzawa M. Correlation of indocyanine green angiography findings and expression of vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. *Jpn J Ophthalmol.* 2000;44:694-695.
11. Schwesinger C, Yee C, Rohan RM, et al. Intrachoroidal neovascularization in transgenic mice overexpressing vascular endothelial growth factor in the retinal pigment epithelium. *Am J Pathol.* 2001;158:1161-1172.
12. Ohno-Matsui K, Morita I, Tombran-Tink J, et al. Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. *J Cell Physiol.* 2001;189:323-333.
13. Ambati J, Gragoudas ES, Miller JW, et al. Transscleral delivery of bioactive protein to the choroid and retina. *Invest Ophthalmol Vis Sci.* 2000;41:1186-1191.
14. Ambati J, Canakis CS, Miller JW, et al. Diffusion of high molecular weight compounds through sclera. *Invest Ophthalmol Vis Sci.* 2000;41:1181-1185.
15. Olsen TW, Aaberg SY, Geroski DH, et al. Human sclera: thickness and surface area. *Am J Ophthalmol.* 1998;125:237-241.
16. Olsen TW, Edelhauser HF, Lim JI, et al. Human scleral permeability. *Invest Ophthalmol Vis Sci.* 1995;36:1893-1903.
17. Boubriak OA, Urban JPG, Akhtar KM, et al. The effect of hydration and matrix composition on solute diffusion in rabbit sclera. *Exp Eye Res.* 2000;71:503-514.
18. Kamei M, Misono K, Lewis H. A study of the ability of tissue plasminogen activator to diffuse into the subretinal space after intravitreal injection in rabbits. *Am J Ophthalmol.* 1999;128:739-746.
19. Dayle H. Transscleral drug delivery for posterior segment disease. *Adv Drug Deliv Rev.* 2001;52:37-48.
20. Lang JC. Ocular drug delivery: conventional ocular formulations. *Adv Drug Deliv Rev.* 1995;16:39-43.
21. Lam TT, Fu J, Tso MO, et al. A histopathologic study of retinal lesions inflicted by transscleral iontophoresis. *Graefes Arch Clin Exp Ophthalmol.* 1991;229:389-394.
22. Carrasquillo KG, Costantino HR, Cordero RA, et al. On the structural preservation of recombinant human growth hormone in a dried film of a synthetic biodegradable polymer. *J Pharm Sci.* 1999;88:166-173.
23. Carrasquillo KG, Carro JC, Alejandro A, et al. Reduction of structural perturbations in bovine serum albumin by non-aqueous microencapsulation. *J Pharm Pharmacol.* 2001;53:115-120.
24. Carrasquillo KG, Stanley AM, Aponte JC, et al. Non-aqueous encapsulation of excipient-stabilized spray freeze-dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein. *J Control Release.* 2001;76:199-208.
25. Gopferich A, Langer R. Modeling monomer release from bioerodible polymers. *J Control Release.* 1995;33:55-69.
26. Batycky RP, Hanes J, Langer R, et al. A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. *J Pharm Sci.* 1997;86:1464-1477.
27. Frazza EJ, Schmitt EE. A new absorbable suture. *J Biomed Mater Res. Symp.* 1971;1:43-58.
28. Hasirci V, Berthiaume F, Bondre SP, et. al. Expression of liver-specific functions by rat hepatocytes seeded in treated poly(lactic-*co*-glycolic) acid biodegradable foams. *Tissue Eng.* 2001;7:385-394.
29. Ma PX, Choi JW. Biodegradable polymer scaffolds with well-defined interconnected spherical pore network. *Tissue Eng.* 2001;7:23-33.
30. Mallery SR, Kang PP, Ness GM, et al. Controlled-release of doxorubicin from poly(lactic-co-glycolic) microspheres significantly enhances cytotoxicity against cultured AIDS-related Kaposi's sarcoma cells. *Anticancer Res.* 2000;20:2817-2825.
31. Moritera T, Ogura Y, Honda Y, et al. Microspheres of biodegradable polymers as a drug-delivery system in the vitreous. *Invest Ophthalmol Vis Sci.* 1991;32:1785-1790.
32. Giordano GG, Chevez-Barrios P, Refojo MF, et al. Biodegradation and tissue reaction to intravitreous biodegradable poly(DL-lactic-co-glycolic)acid microspheres. *Curr Eye Res.* 1995;14:761-768.
33. Fitzwater AM, Polisky B. A SELEX primer. *Methods Enzymol.* 1996;267:275-301.
34. Costantino HR, Carrasquillo KG, Cordero RA, et al. Effect of excipients on the stability and structure of lyophilized recombinant growth hormone. *J Pharm Sci.* 1998;87:1412-1420.
35. Carrasquillo KG, Sanchez C, Griebenow K. Relationship between conformational stability and lyophilization-induced structural changes in chymotrypsin. *Biotech Appl Biochem.* 2000;31:41-53.
36. Jones DS, Barstad PA, Feild MJ, et al. Immunospecific reduction of antioligonucleotide antibody-forming cells with a tetrakis-oligonucleotide conjugate (LJP 394), a therapeutic candidate for the treatment of lupus nephritis. *J Med Chem.* 1995;38:2138-2144.

37. Park TG, Lu W, Crotts G, et al. Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly(D,L-lactic-co-glycolic acid) microspheres. *J Control Release.* 1995;33:211-222.
38. Shelton VM, Sosnick TR, Pan T. Applicability of urea in the thermodynamic analysis of secondary and tertiary RNA folding. *Biochemistry.* 1999;38:16831-16839.
39. Carmona P, Rodríguez-Casado A, Molina M. Conformational structure and binding mode of glyceraldehyde-3-phosphate dehydrogenase to tRNA studied by Raman and CD spectroscopy. *Biochim Biophys Acta.* 1999;1432:222-233.
40. Geroski DH, Edelhauser HF. Drug delivery for posterior segment eye disease. *Invest Ophthalmol Vis Sci.* 2000;41:961-964.
41. Higuchi T. Rate of release of medicaments from ointments base containing drugs in suspension. *J Pharm Sci.* 1961;50:874-875.
42. Higuchi T. Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci.* 1963;52:1145-1149.
43. Noriyouki K, Ogura Y, Yasukawa T, et al. Long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. *J Control Rel.* 2000;68:263-271.
44. Lu B, Wang ZR, Yang H. Long-acting delivery microspheres of levo-norgesterol-poly(3-hydroxybutyrate): their preparation, characterization and contraceptive tests on mice. *J Microencap.* 2001; 18:55-64.
45. Lindahl T. Instability and decay of the primary structure of DNA. *Nature.* 1993;362:709-715.
46. Demple B, Harrison L. Repair of oxidative damage to DNA. *Annu Rev Biochem.* 1994;63:915-948.
47. Evans RK, Xu Z, Bohannon KE, et al. Evaluation of degradation pathways for plasmid DNA in pharmaceutical formulations via accelerated stability studies. *J Pharm Sci.* 2000;89:76-87.
48. Carrasquillo KG, Cordero RA, Ho S, et al. Structure-guided encapsulation of bovine serum albumin in poly(DL-lactic-co-glycolic) acid. *Pharm Pharmacol Commun.* 1998;4:563-571.
49. Schwendeman SP, Cardamone M, Brandon MR, et al. *Stability of Proteins and Their Delivery from Biodegradable Polymer Microspheres.* New York: Marcel Dekker; 1996.
50. Ando S, Putnam D, Pack DW, et al. PLGA microspheres containing plasmid DNA: preservation of supercoiled DNA via cryopreparation and carbohydrate stabilization. *J Pharm Sci.* 1999;88:126-130.
51. Sanchez A, Villamayor B, Guo Y, et al. Formulation strategies for the stabilization of tetanus toxoid in poly(lactide-co-glycolide) microspheres. *Int J Pharmaceutics.* 1999;185:255-266.
52. van Sliedregt A, Radder AM, deGroot K, et al. In vitro biocompatibility testing of polylactides. I. Proliferation of different cell types. *J Mater Sci Mater Med.* 1992;3:365-370.
53. Athanasiou KA, Niederauer GG, Mauli C. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials.* 1996;17:93-102.
54. Prausnitz MR, Edwards A, Noonan JS, et al. Measurement and prediction of transient transport across sclera for drug delivery to the eye. *Ind Eng Chem Res.* 1998;37:2903-2907.
55. Foster CS, Sainz de la Maza M. *The Sclera.* New York: Springer-Verlag; 1994.
56. Haynie GD, D'Amico DJ. *Principles and Practice of Ophthalmology.* Philadelphia: WB Saunders; 1994.

Express Mail Mailing Label No. EV219067000US

PATENT
Attorney Docket No. MEE-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Adamis et al.		
SERIAL NO.:	09/478,099	GROUP NO.:	1632
FILING DATE:	January 5, 2000	EXAMINER:	Anne Marie Falk
TITLE:	Targeted Transscleral Controlled Release Drug Delivery to the Retina and Choroid		

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with the provisions of 37 C.F.R. 1.97 and 1.98, Applicants hereby make of record the patents and publications listed on the accompanying Form PTO-1449, and other information contained herein, for consideration by the Examiner in connection with the examination of the above-identified patent application. Copies of the patents and publications are enclosed.

REMARKS

In accordance with the provisions of 37 C.F.R. 1.97, this statement is being filed (CHECK ONE):

☒ (1) within three (3) months of the **filing date** of a national application other than a continued prosecution application under 37 C.F.R. 1.53(d), or within three (3) months of the **date of entry of the national stage** as set forth in 37 C.F.R. 1.491 in an international application, or before the mailing of the **first Office action** on the merits, or before the mailing of a **first Office action** after the filing of a request for continued examination under 37 C.F.R. 1.114; or

☐ (2) after the period defined in (1) but before the mailing date of a **final action** or a **notice of allowance** under 37 C.F.R. 1.311, and

☐ the requisite Statement is below, **OR**

☐ the requisite fee under 37 C.F.R. 1.17(p), namely **$180.00**, is included herein, or

☐ (3) after the mailing date of a **final action** or **notice of allowance** but before the payment of the **issue fee**, **AND**

☐ the requisite Statement is below, **AND**

☐ the requisite petition fee under 37 C.F.R. 1.17(p), namely **$180.00** is included herein.

It is respectfully requested that each of the patents and publications listed on the attached Form PTO-1449, and other information contained herein, be made of record in this application.

STATEMENT

As required under 37 C.F.R. 1.97(e), Applicant(s), through the undersigned, hereby state either that **[check the appropriate space only if either (2) or (3) is checked on the previous page and the Statement is required]**:

☐ 1. Each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application **not more than three months** prior to the filing of the Information Disclosure Statement; or

☐ 2. No item of information contained in the Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing this Statement after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to **any individual** designated in 37 C.F.R. 1.56(c) **more than three months** prior to the filing of the Information Disclosure Statement.

Respectfully submitted,

D. A. Greenhalgh

Date: August 13, 2004
Reg. No. 38,678

Duncan A. Greenhalgh
Atty/Agent for Applicant(s)
Testa, Hurwitz, & Thibeault, LLP
High Street Tower
125 High Street
Boston, Massachusetts 02110

Tel. No.: (617) 248-7317
Fax No.: (617) 248-7100

Express Mail Mailing Label No. EV219067000US

FORM PTO - 1449 **SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT**	ATTORNEY DOCKET NO.: MEE-005 APPLICANT(S): Adamis et al. SERIAL NO.: 09/478,099 FILING DATE: January 5, 2000 GROUP: 1632

U.S. PATENT DOCUMENTS

EXAM. INIT.		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB CLASS	FILING DATE IF APPROPRIATE
	A100	5,124,155	06/23/92	Reich	424	428	09/17/90
	A101	5,314,419	05/24/94	Pelling	604	294	10/30/92
	A102	2002/0146458 A1	10/10/02	Ogura et al.	424	491	04/05/02

FOREIGN PATENT DOCUMENTS

EXAM. INIT.		DOCUMENT NUMBER	DATE	COUNTRY CODE	CLASS	SUB CLASS	FILING DATE	ABSTRACT ONLY	ENGLISH LANG (Y/N)
	B15	97/35621	10/02/97	WO				Yes (see A102)	Yes
	B16	00/07565	02/17/00	WO					Yes

OTHER ART, JOURNAL ARTICLES, ETC.

EXAM. INIT.	OTHER DOCUMENTS: (Including Author, Title, Date, Relevant Pages, Place of Publication)	
	C24	Friedberg et al., "Device drug delivery to the eye: Collagen shields, iontophoresis, and pumps" Ophthalmology (1991) 5(98):725-732.
	C25	Ho *et al.*, "The MAI hydrophilic implant for scleral buckling: a review" Ophthalmic Surgery (1984) 6(15):511-515.
	C26	Kunou *et al.*, "Biodegradable scleral implant for intraocular controlled delivery of corticosteroid" Investigative Ophthalmology and Visual Science (1996) 3(37):S41.
	C27	Miki *et al.*, "A method for chronic drug infusion into the eye" Japanese Journal of Ophthalmology (1984) 2(28):140-146.
	C28	Nesterov *et al.*, "A new method of drug administration into the posterior section of the sub-Tenon's space" Database Medline Online! US National Library of Science (NLM), Berhesda, MD (09/1991) – abstract only.

EXAMINER	DATE CONSIDERED

3079808

B15

EXPRESS MAIL MAILING LABEL
NO. EV219067000US

XP-002279329

AN - 1997-489398 [45]
AP - [Cont of] WO1997JP00939 19970319; [Cont of] US19980155522 19980925; US20020116874 20020405; JP19960068609 19960325; EP19970908497 19970319; [Based on WO9735621] ; [Div ex] JP19960068609 19960325; JP20000370834 19960325; [Previous Publ. JP9255555]
CPY - SANT
CT - JP558882; JP6312943
DC - A96 B04 B07 D22
DS - AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE LI
DR - 0009-S 0009-U 0448-S 0448-U
FS - CPI
IC - A61K9/00 ; A61K9/16 ; A61K9/50 ; A61K38/00 ; A61K38/21 ; A61K47/30 ; A61K47/34 ; A61P27/02
IN - KUNOU N; OGURA Y; OTA A
MC - A05-E02 A12-V02A B04-C03D B04-N04 B14-N03 D09-C
M1 - [01] H4 H401 H481 H8 J0 J011 J1 J171 M280 M311 M321 M342 M349 M381 M391 M416 M423 M431 M620 M730 M782 M903 M904 M910 N104 N152 P922 V275 V735 V743; R00448-M R00448-Q; 0448-S 0448-U
- [02] H4 H401 H481 H8 J0 J011 J1 J171 M280 M312 M321 M331 M340 M342 M349 M381 M391 M416 M423 M431 M620 M730 M782 M903 M904 M910 N104 N152 P922 V275 V735 V743; R00009-M R00009-Q; 0009-S 0009-U
PA - (SANT) SANTEN PHARM CO LTD
PN - US2002146458 A1 20021010 DW200269 A61K38/21 000pp
- WO9735621 A1 19971002 DW199745 A61K47/30 Jpn 017pp
- JP9255555 A 19970930 DW199749 A61K9/00 005pp
- EP0904787 A1 19990331 DW199917 A61K47/30 Eng 000pp
- JP2001151693 A 20010605 DW200138 A61K38/21 005pp
- JP3309175B2 B2 20020729 DW200256 A61K9/00 005pp
PR - JP19960068609 19960325; JP20000370834 19960325
XA - C1997-155962
XIC - A61K-009/00 ; A61K-009/16 ; A61K-009/50 ; A61K-038/00 ; A61K-038/21 ; A61K-047/30 ; A61K-047/34 ; A61P-027/02
AB - WO9735621 A composition (I) comprises a protein material adsorbed onto the surface of microfine grains of lactic acid copolymer. Also claimed is a scleral plug prepared using (I).
- USE - The scleral plug is used in the prevention and treatment of intraocular diseases, as a means for releasing medicaments (e.g. interferon- beta and IFN- beta), in the extraction of the vitreous body or in the treatment of retinal disease (e.g. diseases associated with diabetes, macular degeneration, or vascular occlusion) (all claimed).
- ADVANTAGE - The protein is protected from loss of activity due to heat or organic solvent.
- (Dwg.0/0)
CN - R00448-M R00448-Q R00009-M R00009-Q
DN - CA CN KR NO US
DRL - 0448-S 0448-U 0009-S 0009-U
IW - PROTEIN ADSORB LACTATE COPOLYMER GRAIN SCLERAL PLUG INTRA=OCULAR SURGICAL TREAT RETINA DISEASE RELEASE MEDICAMENT EXTRACT VITREOUS MATTER
IKW - PROTEIN ADSORB LACTATE COPOLYMER GRAIN SCLERAL PLUG INTRA=OCULAR

SURGICAL TREAT RETINA DISEASE RELEASE MEDICAMENT EXTRACT VITREOUS MATTER

INW - KUNOU N; OGURA Y; OTA A

NC - 024

OPD - 1996-03-25

ORD - 1997-09-30

PAW - (SANT) SANTEN PHARM CO LTD

TI - Protein adsorbed onto lactate copolymer grains used as scleral plug for intra=ocular surgery - during treatment of retinal disease, release of medicaments and extraction of vitreous matter

USAB- US2002146458 A composition (I) comprises a protein material adsorbed onto the surface of microfine grains of lactic acid copolymer. Also claimed is a scleral plug prepared using (I).

- USE - The scleral plug is used in the prevention and treatment of intraocular diseases, as a means for releasing medicaments (e.g. interferon- beta and IFN- beta), in the extraction of the vitreous body or in the treatment of retinal disease (e.g. diseases associated with diabetes, macular degeneration, or vascular occlusion) (all claimed).

- ADVANTAGE - The protein is protected from loss of activity due to heat or organic solvent.

A01 - [001] 018 ; R00009 G2108 D01 D11 D10 D50 D60 D83 F27 F26 F36 F35 ; R00448 G2108 D01 D11 D10 D50 D60 D82 F27 F26 F36 F35 ; H0022 H0011 ; P1978-R P0839 D01 D50 D63 F41 ; S9999 S1456-R ;

- [002] 018 ; ND01 ; Q9999 Q8048 Q7987 ; Q9999 Q8037 Q7987 ; Q9999 Q7250 ; B9999 B5209 B5185 B4740 ; B9999 B5094 B4977 B4740 ;

B16

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 9/00, 38/55, 38/46, 48/00, 39/395, A61P 21/02

A2

(11) International Publication Number: WO 00/07565

(43) International Publication Date: 17 February 2000 (17.02.00)

(21) International Application Number: PCT/US99/17543

(22) International Filing Date: 2 August 1999 (02.08.99)

(30) Priority Data:
09/127,920 3 August 1998 (03.08.98) US

(71) Applicant: INSITE VISION, INCORPORATED [US/US]; 965 Atlantic Avenue, Alameda, CA 94588 (US).

(72) Inventors: BOWMAN, Lyle, M.; 5135 Mount Tam Circle, Pleasant, CA 94588 (US). PFEIFFER, James, F.; 2369 Thackery Avenue, Oakland, CA 94611 (US). CLARK, Leslie, A.; 829 #B Lincoln Avenue, Alameda, CA 94501 (US). HECKER, Karl, I.; 400 Hurricane Road, Keene, NH 03431–2161 (US).

(74) Agents: FREED, Joel, M. et al.; Howrey & Simon, 1299 Pennsylvania Avenue, N.W., Box 34, Washington, DC 20004–2402 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS OF OPHTHALMIC ADMINISTRATION

(57) Abstract

Intrascleral injection of a therapeutic or diagnostic material at a location overlying the retina provides a minimally invasive technique for delivering the agent to the posterior segment of the eye. The procedure also allows for close proximity of the material to the targeted site and can be effectively used to treat conditions associated with the posterior segment of the eye, including macular degeneration, vein occlusion, and diabetic retinopathy. The sclera can be used to hold a depot of the material such as for sustained released or as a conduit for propelling material through whereby the material is delivered immediately to the underlying tissues but without physically penetrating the sclera with an instrument or otherwise unreasonably traumatizing the eye.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

METHODS OF OPHTHALMIC ADMINISTRATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to methods of ophthalmic administration. Specifically, the methods relate to intrascleral injection of therapeutic or diagnostic materials.

2. Description of the Related Art

Delivering therapeutic or diagnostic agents to the posterior segment of the eye, especially to the retina, macula, etc., poses several challenges. Topical instillation of an agent to the front of the eye such as by eye drops, generally provides low amounts of the agent (including none) to the posterior portion of the eye, due in part to poor diffusion through the various layers as well as the natural clearing processes encountered. Providing effective amounts of an agent to, for example, the retina via topical instillation is generally not possible given the distance and number of layers between the deposit site of the agent and the site to be treated. Another potential shortcoming with topical instillation is that the composition tends to be quickly removed from the eye by tears and other natural clearing processes. The resulting short duration of contact can further limit the likelihood of an appreciable amount of the agent reaching the posterior segment.

Conversely, systemic delivery of an agent to the posterior segment of the eye such as by oral administration, is limited by the blood-retinal barrier. The barrier limits the size and amount of agents that can reach the choroid and retina. Moreover, because the agent is systemically delivered, the dosage is limited so as not to provide a toxic dose of the agent to other parts of the body. This is especially a concern in treating chronic disorders where a long term dosing regimen is typically required. For this reason,

overcoming the barrier by administering higher doses of the agent is usually not a practical alternative. Likewise, the risk of side effects is increased with systemic delivery.

Other proposals for delivering agents to the eye include the use of inserts and implants which release the agent over time onto or into the eye. An insert, as used in this application, is a device inserted over the eye, such as on the conjunctival layer, and generally comprises a polymer matrix containing an active agent. The agent that is released from the insert can diffuse through the sclera and into the eye. While sustained or long term agent contact with the eye can be achieved by this method, little if any of the agent reaches the posterior segment of the eye for much the same reasons as topical instillation. Implants are devices similar to inserts but they are surgically placed within the eye. Accordingly, implants bring the risk of infection and other problems due to its more invasive nature.

For example, U.S. patent 4,863,457 to Lee relates to an implant having a stem and base wherein the stem releases a drug and is positioned to extend into a canal, passageway, or orifice of the eye. The implant is taught to serve two functions: internal delivery of drug and mechanical prevention of passageway closure. The drawings illustrate placing the base of the implant in the subconjunctival space, or within the sclera itself, with the stem extending into the anterior chamber. The implant is taught to be especially useful in post-operative glaucoma patients as the drugs released can suppress scar tissue around the stem while the stem structure helps to maintain a passageway from the anterior chamber to Schlemm's canal. In this way, the implant is taught to ensure continued drainage of the aqueous humor from the anterior chamber and prevent a recurrence of the pressure buildup caused by glaucoma.

However, this implant is directed to treating the anterior chamber and not the posterior segment of the eye. Indeed, the option of inserting the implant into the sclera is problematic if attempted in the posterior segment of the eye. Here, partially

cutting the sclera where it overlies the retina and inserting the base and stem of the implant therein, raises the risk of retinal detachment and choroidal hemorrhage. Only in the front of the eye, where the sclera does not overlie the retina and the vasculature of the choroid is low, can the partial thickness sclera flap technique be practically performed. Accordingly, the design and placement of this implant is not effective for delivering an agent to the posterior portion of the eye.

U.S. patent 5,707,643 to Ogura *et al.* relates to a biodegradable scleral plug that is inserted through an incision in the sclera into the vitreous body. The plug releases a drug into the vitreous body for treating the retina. The path of the plug is not, however, indicated. Assumedly, the plug would extend through the avascular region of the pars plana so as not to rupture any significant blood vessels or the retina. The drug will be applied to the entire retina by diffusion through the vitreous body, thus precluding the ability to provide a more concentrated application of the drug to one portion of the retina. Also, the invasive nature of the plug brings various risks including the risk of infection.

U.S. patent 5,443,505 to Wong *et al.* relates to implants that are taught to deliver drug to a localized site. The implants are typically placed in the suprachoroidal space over an avascular region of the eye such as the pars plana or a surgically induced avascular region. Another embodiment involves forming a partial thickness scleral flap over an avascular region, inserting the implant onto the remaining scleral bed, optionally with holes therein, and suturing closed the flap. The drug diffuses into the vitreous region and the intraocular structure. Locating the implant close to the back of the eye is apparently not possible as the region would not be avascular, unless surgery is performed to make an avascular region. Such removal is normally undesirable since vision loss will be induced.

Another delivery approach is direct injection. For the posterior segment of the eye, an intravitreal injection has been used to deliver drugs into the vitreous body. U.S. patent 5,632,984 to Wong *et al.* relates to the treatment of macular degeneration with various drugs by intraocular injection. The drugs are preferably injected as microcapsules. The intraocular injection into the posterior segment is taught to allow diffusion of the drug throughout the vitreous, the entire retina, the choroid and the opposing sclera. Similarly, U.S. patent 5,770,589 to Billson *et al.* relates to treating macular degeneration by intravitreally injecting an anti-inflammatory into the vitreous humor. These invasive injections are normally administered through the pars plana in order to minimize the damage to the eye. While drug is delivered to the posterior segment, it is not specifically administered to a target area such as the macula, but rather is supplied to the entire posterior segment. Additionally, the procedure has a high risk of infection and retinal detachment and has restricted use.

U.S. patent 5,767,079 to Glaser *et al.* relates to the treatment of ophthalmic disorders including macular holes and macular degeneration, by administration of TGF-ß. The method of administration varies depending upon the nature and location of the pathology. The patent contemplates placing an effective amount of the growth factor on the ophthalmic abnormality. In treating the macula and retina, the examples teach that a surgical procedure involving a core vitrectomy or a complete pars plana vitrectomy is performed before the growth factor can be directly applied. The patent does mention the possible use of, *inter alia*, an intrascleral injection. However, no specifics are given about such a procedure, nor is such a procedure well known in the art. Presumably, the patentee intended either administration to the sclera on the anterior segment of the eye at an avascular region or administration to the sclera behind the retina via a surgical procedure through the vitreous body, retina, and choroid. The former method will not provide a large amount of drug to the posterior segment, as discussed above with regard to topical

instillation and implants. The latter method is a dramatic, highly invasive technique that would be suitable only where partial vision loss has already occurred or was imminently threatened. Such a procedure carries a high risk of infection or retinal detachment as well as loss of vision and clearly is problematic for chronic administration.

U.S. patent 5,273,530 to del Cerro *et al.* relates to the intraretinal delivery and withdrawal of samples and a device therefor. Unlike direct intraocular injection techniques, the method disclosed in this patent avoids the use of a pars plana incision and instead uses an insertion path around the exterior of the orbit. The device, having a curved handle and a tip with collar, allows a cannula to be inserted through the posterior sclera and down into the subretinal space without passing through the vitreous body. The collar is stated to regulate the penetration to the desired depth. The method is basically directed to supplying cells to and/or withdrawing samples from the subretinal space. However, the device is taught to be adjustable to any part of the eye including the scleral area, the choroidal area, the subretinal area, the retinal area and the vitreous area. In use, the disclosed subretinal delivery method presents a significant risk of causing choroidal hemorrhaging. It should be noted that although the approximate location of the cannula can be observed through a slit lamp by tinting, the penetration of the cannula through the sclera and choroid can not be seen until the tip of the cannula penetrates the retinal surface.

The above methods show that delivering agents to the posterior segment of the eye, especially the back of the eye at the retina, macula, etc., is difficult. This region of the eye is isolated by both the anterior segment and the blood-retinal barrier. The techniques which are relatively easy to apply (topical instillation, oral administration) generally do not deliver a sufficient amount of the agent to the posterior segment and/or present toxicity or side effect problems. In contrast, techniques that deliver effective amounts (intravitreal injection) are complicated, invasive procedures that subject the

patient to the risk of infection, retinal detachment, and further vision or eye damage. A minimally invasive method for delivering agents to the posterior segment of the eye would be of great benefit.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for administering agents to the eye.

It is another object of the present invention to provide a method for administering agents to the eye that is minimally invasive.

Preferred forms of the invention contemplated accomplish at least one of the above objects. One embodiment of the invention is a method of intrascleral injection, which comprises injecting into the scleral layer of an eye through a location on the exterior surface of the sclera which overlies retinal tissue an effective amount of a therapeutic or diagnostic material. Depending on the injection conditions, the material will (1) form a depot within the scleral layer and diffuse into the underlying tissue layers such as the choroid and/or retina, (2) be propelled through the scleral layer and into the underlying layers, or (3) a combination of both (1) and (2). By entering the sclera from the external side, the method avoids the invasiveness of the intravitreal injection technique, thereby reducing the risk of infection and allowing a regimen of treatments to be given throughout the year, if needed. Also, because the sclera moves with the entire eye including the retina, the site of deposit on the sclera will map to the corresponding point on the underlying retina, even as the eye moves within the eye socket. This means that site specific delivery can be achieved and maintained. Thus, by depositing material into the sclera at a site overlying the macula, the material will be easily delivered to the macula and surrounding tissues.

The injection procedure is not particularly limited and embraces the use of a cannula or needle as well as needle-less particle/solution techniques. In a preferred embodiment, a cannula is inserted into the sclera in a rotational direction relative to the eye and not orthogonal to the surface of the sclera. By angling the cannula insertion into the sclera, the risk of accidentally perforating the sclera and causing damage to the underlying tissue (choroid and/retina) or hemorrhaging can be reduced or eliminated.

The present invention allows the delivery of a variety of agents to the posterior segment of the eye whenever such delivery would be desirable, including treating conditions of the posterior or anterior segments and diagnosing various conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates an eye having a cannula inserted into the scleral in accordance with one embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves injecting a material into the sclera of an eye. The eye can be of any vertebrate animal including human and non-human mammals. The sclera is a thin, avascular layer, comprised of a highly ordered collagen network, that surrounds most of the vertebrate eye. The cornea replaces the sclera in the front of the eye, with the transition from sclera to cornea occurring at the limbus. Because the sclera is avascular, there is essentially no risk of hemorrhaging after an injection therein and the injected material is not rapidly removed or "cleared" from the eye. Thus the sclera can be effectively utilized in the present invention as a natural storage depot. Further, because of the fibrous and inelastic nature of the sclera, it can be used as a diffuser screen through which the material is propelled. This allows for the rapid delivery of the material to the underlying tissue(s). In any event, injecting the material into the scleral layer, either so it

can diffuse out or so it is propelled out, provides for a minimally invasive technique for providing the material to ocular tissues in the posterior segment of the eye.

The material can be placed within the sclera by any suitable injection technique including cannular and non-cannular techniques. Typically a cannula is inserted into the sclera and the therapeutic or diagnostic material is then injected through the cannula and into the scleral layer. In more detail, an embodiment of the present invention using a cannula is described below with reference to fig. 1.

Eye 10 (not drawn to scale) can be divided into an anterior segment 20 and posterior segment 30 with an equator 40. Sclera 11 covers the outside of the eye around the posterior segment and part of the anterior segment while cornea 21 covers the outer part of the remainder of the anterior segment. Underlying the sclera is choroid 12 and retina 13. A cannula 50 (shown here as a needle with a beveled terminal end) has been inserted into the sclera from a location on the external (or outer) surface of the sclera that overlies the retina. In this inserted position, conveying material through cannula 50 will result in injecting the material into the scleral layer. The cannula has been inserted in a substantially rotational direction meaning that the insertion path into the sclera generally points around the eye and not into the center of the eye. This is a preferred embodiment because it decreases the risk of accidentally penetrating through the sclera and into the choroid or retina. Obviously, inserting a cannula, especially a sharpened or beveled cannula, into the vascular choroid or light sensitive retina can cause serious injury to these layers with resulting vision impairment. Generally insertion of the cannula in a "substantially rotational direction" will be performed at an insertion angle of less than about 60 degrees; the "insertion angle" being defined by the angle formed between the tangent to the sclera at the external point of entry and the insertion path of the cannula into the sclera (or in the case of a curved cannula, the tangent to the curved cannula insertion path at the entry point into the sclera). Preferably the insertion angle is less than

50, more preferably from about 20 to about 40 degrees. In one embodiment the insertion angle is about 30 degrees.

In a preferred embodiment, the cannula is inserted in an orientation to the sclera such that it must exit the sclera, if at all, through another location on the exterior surface of the sclera: hereinafter a "fail safe orientation." This can be accomplished, for example, by inserting a straight cannula at a sufficiently low insertion angle. Because the sclera is curved, the cannula can be angled so as to travel on a path that is tangential to a point on the curving interior surface layer of the sclera. At all insertion angles lower than this tangential angle, the closest the cannula will come to the inner surface of the sclera is a point above the tangent point. Further extending the cannula beyond this point will bring the leading end of the cannula further away from the interior surface of the sclera and closer to the exterior surface of the sclera. Thus, the cannula will miss perforating the interior surface of the sclera no matter how far the cannula is extended. This provides a safer injection technique in that if the cannula is inserted "too far," the result is only that the injected material will not go into the sclera as intended, but instead will be deposited on the exterior of the sclera. Such injections into the external part of the eye should have no deleterious effect. Orienting the cannula, whether curved or straight, such that it enters the sclera from the exterior surface thereof and must exit the sclera, if at all, also through the exterior surface and not through the inner surface of the sclera, significantly reduces the risk of accidentally damaging the choroid or retina.

Inserting the cannula in a substantially rotational direction also allows for increased insertion distances of the cannula into the sclera which can increase the hydrodynamic seal between the cannula and the scleral tissue. A poor hydrodynamic seal can lead to the material leaking out of the sclera along the sides of the cannula during the injection. Generally, the insertion distance of the cannula into the scleral layer is at least about 1.5 mm, more typically about 2-3 mm, in order to have a good hydrodynamic seal,

although the insertion distance is not limited thereto. In some embodiments, the cannula is preferably inserted into the sclera a distance that is greater than or equal to the thickness of the sclera, preferably at least one and a half times the thickness, measured at the entry point on the exterior surface. This is particularly useful near the equator where the sclera is quite thin, but is not limited to such a region. By increasing the surface area between the cannula and the scleral tissue, a better hydrodynamic seal can be formed which allows for larger and/or faster injections.

The cannula is not particularly limited and need only fit within the thickness of the sclera at the point of entry. Preferably the cannula is sufficiently small in diameter that no hole is visible in the sclera upon macroscopic observation of the entry site after the injection. Typically, at least a 25 gauge, preferably at least a 28 gauge, more preferably about 30-33 gauge cannula is employed, but such is not required. The size of the cannula depends in part on the viscosity of the material to be injected, the amount of material to be injected, and the time of injection. A very fine gauge cannula, while causing little if any trauma to the eye, may not be able to allow sufficient flow of a particular material into the sclera and thus would not serve as a useful conduit. The above gauge sizes are, especially for humans, a size that typically accommodates these competing features. Preferably the cannula is sharp on its leading end (i.e. a needle) such as with a bevel or a hollow ground point. In some cases it may be desirable that the bevel engages the sclera in an upside down orientation whereby the leading edge of the bevel is adjacent to and makes first contact with the exterior surface of the sclera. This can aid in holding the cannula in alignment with the sclera as the cannula is inserted and in preventing or reducing the risk of the cannula rotating the eye or sliding/skidding on the scleral tissue. The desirability of such an upside down bevel approach depends upon the insertion angle, the thickness of the sclera, the shape of the cannula and the size of the cannula. The aperture through which the material exists the cannula can be on the terminal end of the cannula or on the

side of the cannula. By selecting the aperture location, the general direction of the material being injected into the sclera can be controlled.

The insertion of the cannula can be carried out by hand or with a device. In a preferred embodiment of the invention, the insertion is carried out using a guided injection device. In general, a guided injection device has a mechanism for providing a needle at a predetermined angle of insertion and preferably a predetermined depth of insertion. For example, a device having a guide platform which comprises a support surface that generally conforms to the shape of the sclera and a channel extending through it for guiding the needle can be advantageously employed. When the support surface is contacted to the exterior surface of the sclera, the angle of the channel relative to the sclera is fixed. The needle transmitted through the channel will thus be inserted into the sclera at the predetermined insertion angle. Various mechanical means, such as a stop or collar, can be used to limit the insertion distance of the needle. The needle itself is connected to the material to be injected, such as by directly attaching to a reservoir on the device or to a remote reservoir, so as to facilitate the injection step. For safety the needle is preferably retractable such that after injection of the material, the needle can be withdrawn back within the device, behind the support surface. Actuators for achieving the back and forth movement of a needle are well known in the art. Such a guide platform can be placed on the distal end of the device shown in U.S. patent 5,273,530, and thus, with an appropriate predetermined angle of insertion, make the blind insertion of a needle into the sclera at the back of the eye a safe procedure.

With the cannula inserted into the sclera, the material is injected via the cannula and into the scleral layer. The rate of injection of the material via a cannula into the sclera is dependent on several factors including the viscosity of the material, the duration of the injection (or the amount of material to be injected), and the desired result of the injection and can be readily determined by workers of ordinary skill in the art.

Generally, the injection rate is at least about 0.1 µl/s, typically from about 0.1 to about 8.0 µl/s, although higher rates may be preferred for some applications. For example, if the injection is intended to form a depot of material within the sclera so as to accommodate extended release of the material to the underlying tissues, then generally lower injections rates are used such as from 0.1 to about 3.0 µl/s. In this way, the injected material is held in the scleral layer and diffuses out over time. Alternatively, the material can be injected into the sclera so as to leave more quickly than by simple diffusion. By injecting the material under sufficient force, at least a portion of the material can be propelled through the scleral layer. Under this approach, generally higher injection rates are used such as from 2.0 to about 8.0 µl/s. However, it is possible to propel a portion of the injected material through the sclera without using high flow rates by injecting larger amounts of material than the sclera can hold. In this way the storage depot capability of the sclera is exceeded and the injected material is forced out and into the underlying tissues. Nonetheless, usually the injection rate is within the above range and the injected material is propelled through the sclera by the force of injection (i.e., the velocity obtained therefrom), only. Such higher injection forces can be used without a high risk of damage to the underlying sensitive tissues because, in part, the partial thickness of the scleral layer serves as a diffuser to slow down and somewhat disperse the injection stream. In either event, the injection conditions are such that at least a portion of the material injected into the scleral layer is also propelled through the remainder of the scleral layer and into the underlying tissues (hereinafter sometimes referred to as the "propelling-type injection"). The amount of propelled material that exits the sclera varies from greater than zero to essentially all of the injected material. Typically, at least 10 %, more preferably at least about 25%, is propelled through the scleral layer and into the underlying tissues. The remainder of the material will usually diffuse out of the sclera over time, although such is not required. In this way, a combination of immediate or near immediate treatment and

extended or sustained treatment can be attained by appropriately proportioning the amount of material that is propelled out of the sclera and the amount that diffuses out of the sclera. Other factors that affect the propelling/diffusing ratio include the nature and viscosity of the material, the proximity of the cannula to the interior surface of the sclera, the angle of injection and the orientation of the cannula aperture, and the amount of material injected.

In a preferred embodiment for a propelling-type injection, the cannula aperture is located on the side, generally perpendicular to the insertion direction, and oriented toward the interior surface of the sclera. In this way, the injected material is directed toward the underlying ocular tissues and is thus more likely to have sufficient propulsion from the injection to traverse the remaining scleral layer and reach these tissues.

One advantage of the propelling-type injection is the ability to force relatively large particles through the scleral layer that would not ordinarily or not readily diffuse out of the sclera. This means that proteins, viral vectors, antibodies, gene therapy constructs, etc., can be delivered to the ocular tissues in the posterior segment of the eye without penetrating the sclera and despite the fact that the materials may be too big to fit through the scleral layer. The sclera, although inelastic, will nonetheless allow these larger particles to pass through. Typically these larger particles have a particle size of at least 50 nanometers and generally are in the range of 50 to 200 manometers, preferably 50 to 150 nanometers. In this embodiment, the hydrodynamic seal around the cannula should be taken into account along with the injection rate in order to prevent the injected material from backing out of the sclera along the sides of the cannula, as has been discussed above.

Cannular injections typically will last for up to 10 seconds, although longer injection times are possible, especially with the use of a hand rest/support, and are contemplated by the present invention.

A different embodiment of the present invention uses needle-less injection to carry out the injection of material into the cannula. Needle-less injection techniques are well known in connection with injecting into skin. In general, a solution or dry particles are driven by a forced fluid such as gas at very high velocities to the surface of the skin. The duration of the injection is very brief, fractions of a second, so as to be considered instantaneous. At the point of contact (the site of injection), the nozzle through which the material is propelled, forms a seal with the skin. Because of the high speed, generally supersonic, the material penetrates the skin, the path of least resistance, without the aid of a needle. However, skin is relatively elastic and unlike the sclera. Accordingly, devices commercially sold for needle-less injection into skin will generally have poor performance if used to inject into the sclera. In particular, a short burst of material directed at high speed at the sclera tends to bounce off instead of penetrate the scleral layer. Accordingly, a slower, sub-sonic approach with longer injection duration is preferred for intrascleral injection. By driving the material so as to attain sufficient momentum, but without excess speed, the material will be more likely to penetrate into the scleral layer than bounce off. Typically the injection duration is at least one second and generally from 1 to 10 seconds, although longer times can be used. Improving the seal between the nozzle and the sclera is also beneficial to improving penetration efficiency.

As with a cannular injection, the material injected by the needle-less technique can form a depot within the sclera and/or a portion of the material can be propelled through the sclera and into the underlying tissues. The depth of penetration depends on the size and nature of the material, the momentum of the material, the duration of the injection and the seal between the nozzle and the sclera. Unlike the cannular propelling-type injection, needle-less injection generally can not be used to inject large particles. It is believed that this is do in part to (1) the nature and physical dynamics of needle-less injection technology and (2) the fact that the needle-less injection technique

requires the material to be propelled the entire thickness of the sclera in order to reach the underlying tissues. In contrast, the cannular propelling-type injection propels the material from within the scleral layer and thus need only propel the material a partial thickness of the sclera; i.e. the remaining thickness from the cannular aperture to the interior surface layer. Accordingly, needle-less injection into the sclera normally uses material that has a particle size of less than about 40 nanometers and preferably 20-40 nanometers.

The entry point on the exterior surface of the sclera overlies the retina and thus is in the posterior segment of the eye. It should be noted, however, that the injection of material into the sclera may occur at a location within the sclera that does not overlie the retina, depending upon the angle and direction of injection; e.g., in the case of a cannular injection in a substantially rotational direction, the injection site within the sclera may be anterior to the retina. Nonetheless, generally, and preferably, the injection site of the material within the sclera is also over the retina.

Preferably the entry point and injection site are posterior to the area of eye muscle insertion, more preferably posterior to the equator of the eye, and more preferably more than 45 degrees posterior to the equator. Also, when a disease or condition is present or concentrated in a local area, such as macular degeneration, it is preferred to make the injection in the vicinity of the affected area. In this way, any depot of the injected material formed within the sclera is near the site to be treated while any propelled material is likely to reach the intended affected tissue. Preferably a portion of the injected material at least partially overlies the localized area to be treated. Such can allow for more effective treatment and/or reduced amounts of material needed to be injected. Also, because any material that is not propelled is stored within the sclera, the material remains in proximity with the affected area regardless of eye movement.

The posterior segment can be reached in order to make an injection in a number of ways. The eye can be rotated in order to expose the posterior segment. This is

typically accomplished by holding the conjunctiva with forceps and rotating the eye so that the front of the eye moves downwardly (i.e. "rotated forwardly"). The eye can be rotated in other directions as appropriate and other techniques for rotation can be used as desired. Another technique involves using a curved handled device that can be inserted around the eye to position the cannula, needle-less injection nozzle, or other injection apparatus over the desired posterior location. The concept of such a device is shown in U.S. patent 5,273,530. While the device could be used as shown therein, it should preferably be modified so that the cannula is retractable and more preferably modified so that the cannula will be inserted in a substantially rotational direction.

Accessing the posterior of an eye also normally entails penetrating the conjunctiva. One way is to make an incision in the conjunctiva and insert the cannula, nozzle, or other injection apparatus through the incision to the sclera. Such a method works with both the eye rotating technique and the curved handle device technique discussed above for accessing the posterior segment of the eye. Such an incision is relatively non-invasive and is similar to conjunctival incisions (peritomy) that ophthalmologists make in carrying out other procedures. Another approach is to rotate the eye into the desired position and then inject through the conjunctiva and into the sclera. For example, in the case of a cannular injection, this means inserting the terminal end of the cannula through the conjunctival layer and into the sclera. In this approach, it may be necessary to prevent movement of the conjunctiva relative to the sclera. This can be done by taking into account the relative looseness of the conjunctiva, the angle of insertion, the presence of a bevel and its orientation, and the use of conjunctiva holding or stabilizing devices or techniques. For example, holding a portion of the conjunctiva in place by physical restraint (e.g., friction or pins) while inserting the cannula can be effective in preventing relative movement between the conjunctiva and the sclera. Relative

slipping between the conjunctiva and the sclera are less of a concern with needle-less injection techniques.

The material to be injected can be any material having a therapeutic or diagnostic utility or purpose. The material can be a gas, a liquid, a suspension, a colloidal suspension (particles of less than about 200 nanometers), an emulsion, a gel-sol, a powder, etc., so long as it is injectable. Preferably, the material is injectable through a cannula. Typically the materials injected in the present invention are similar to intravitreal and intramuscular injection formulations in terms of concentrations, viscosities, adjuvants, etc., although such is not required. A large number of diagnostic and therapeutic materials are well known in the art for treating various ocular diseases and conditions, as is their preparation and formulation, and all such materials are specifically contemplated for use in the present invention.

A "therapeutic material" means a material that provides a healing, restraining or prophylactic effect to a disease or condition or which suppresses, ameliorates or prevents the symptoms associated with a disease or condition. The material can be a single substance or a combination of substances. Typically, a therapeutic material is a composition containing a pharmaceutically active agent and an ophthalmically acceptable carrier or diluent. The active agent useful in the present invention include all ophthalmically effective agents, examples of which include anti-angiogenesis agents such as metalloproteinase inhibitors, vascular endothelium growth factor (VEGF) regulating agents, fibroblast growth factor (FGF) regulating agents, integrin blockers, protein kinase C inhibitors, and endogenous angiogenesis inhibitors (e.g., angiostatin); ischemic/reperfusion preventing agents such as NMDA receptor antagonists, AMPA receptor antagonists, antioxidants, peroxidation inhibitors, apoptosis inhibitors, adenosine or adenosine regulating agents, calcium channel blockers, and nitric oxide regulating agents; anti-inflammatory agents such as steroidal and non-steroidal anti-inflammatory

agents; antiviral agents; antioxidants; antibiotics; antitumor agents such as tumor necrosis factors; anti-cataract agents; anti-glaucoma agents; anesthetics; cellular regeneration agents such as telomerase; gene therapy compositions which are typically comprised of a nucleic acid and/or a protein and a vector and include triplex nucleic acids, ribozymes viruses, plasmids, and liposomes; antibodies and fragments thereof; and antisense compounds.

Specific examples of useful active agents include, but are not limited to, pilocarpine, timolol, atenolol, betaxolol, levobunolol, tetracycline, hydrocortisone, prednisolone, prednisone, dexamethasone, progesterone, fluorometholone, lazaroids and 21-aminosteroid compounds as disclosed in U.S. Patent 5,124,154 (incorporated herein by reference), aspirin, benoxaprofen, benzofenac, bucloxic acid, butibufen, carprofen, cicloprofen, cinmetacin, clidanac, clopirac, diclofenac, etodolac, fenbufen, fenclofenac, fenclorac, fenoprofen, fentiazac, flunoxaprofen, furaprofen, flurbiprofen, furobufen, furofenac, ibuprofen, ibufenac, indomethacin, indoprofen, isoxepac, ketorolac, ketroprofen, lactorolac, lonazolac, metiazinic, miroprofen, naproxen, oxaprozin, oxepinac, phenacetin, pirprofen, pirazolac, protizinic acid, sulindac, suprofen, tiaprofenic acid, tolmetin, zomepirac, tolrestat, lisinopril, statil, retinoic acid, methotrexate, mitomycin, urokinase, streptokinase, cephaloridine, chloramphenicol, clindamycin, tobramycin, penicillin, ampicillin, erythromycin, streptomycin, neomycin, cyclosporine A, cyclosporine G, TGF-β, TGF-β2, TNF-α, TNF-β, bFGF, and α-2a interferon, anti-FGF antibody, anti-VEGF antibody, FGF antisense, VEGF antisense, VEGF receptor blockers, cysteine analogs, terilazad mesylate, angiostatin, endostatin, memantine, Cerestat, Batimastat, Marimastat, superoxide dismutase, GEM-antisense compounds, Lexipafant, nanoparticles, adeno viral vectors, adeno-associated viruses, retrovirus vectors, picorna viral vectors, liposomes, cationic lipid systems and protein/nucleic acid complexes.

One advantage of the present invention is the ability to use enzyme-unstable agents. Because the sclera is avascular, enzymes that would normally attack and degrade certain proteins and other agents if placed intraocularly, will not generally reach the intrascleral depot formed by the present invention.

The active agent can be combined with a suitable carrier or diluent, if needed, as is well known in the art and includes aqueous as well as non-aqueous systems. The composition used in the present invention contains no physiologically or ophthalmically harmful constituents. Typically purified or deionized water is used. The pH is adjusted as needed by adding any physiologically and ophthalmically acceptable pH adjusting acids, bases or buffers. Examples of acids include acetic, boric, citric, lactic, phosphoric, hydrochloric, and the like, and examples of bases include sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate, tromethamine, THAM (trishydroxymethylamino-methane), and the like. Salts and buffers would include citrate/dextrose, sodium bicarbonate, ammonium chloride and mixtures of the aforementioned acids and bases. The pH is typically in the neutral range such as from about 6 to about 8, but is not limited thereto. Non-aqueous systems include the use of known ophthalmically acceptable oils such as polyethylene glycols and silicone oils. The active agent can be in solution, in suspension, or both. If the active agent is in solid form, its particle size should be sufficiently limited to permit injection (e.g., the agent is able to pass through a cannula) and so as not to cause irritation to the eye once injected.

In a preferred embodiment, the composition contains a component that facilitates or improves the sustained release of the active agent as is known in the art. For example, incorporating a polymeric suspending agent can provide or enhance sustained release. The polymer should be biodegradable or biocompatible such that it can be cleared from the eye by natural transport effects. The active ingredient can be incorporated into the polymer matrix, adsorbed on the polymer surface, encapsulated within a polymer

coating, etc. as are well known in the art. Examples of suitable polymers include water-soluble polymers such as dextran, polyethylene glycols, polyvinylpyrolidones, polysaccaride gels, Gelrite®, cellulosic polymers like hydroxypropyl methylcellulose and carboxymethylcellulose, hyaluronic acid polymers, and poly(lactic acid) and copolymers of lactic acid and one or more of glycolic acid, malic acid, glyceric acid, and tartaric acid. Carboxy-containing polymers such as uncrosslinked polyacrylic acids and copolymers thereof are also useful as suspending agents for insuring sustained release. Crosslinking is permissible only to the extent that the polymer can clear; i.e., crosslinking generally prevents biodegredation and thus the entire polymer must be susceptible of being cleared from the eye. Other forms include liposomes and lipid emulsions.

The composition should contain a sufficient amount of active ingredient to achieve the desired effect as can be readily determined by workers skilled in the art. In general, the solubility of the active ingredient in water and the concentration of the active ingredient needed in the tissue, guide the amount and rate of release of the agent. It should be borne in mind that the sclera is a depot of limited size and the concentration of the agent may need to reflect this. In general the amount of material to be injected is at least 0.1 µl, typically from around 0.1 to 25 µl, more typically from about 1 to 25 µl, such as from about 3 to about 25 µl or from about 3 to 10 µl. If more material is needed then can be practically delivered in a single injection, then multiple injections can be performed; i.e., injecting 6 µl of therapeutic material in three different sites within the sclera during a single office visit.

Other components of the therapeutic material include solubilizers, stabilizers, preservatives, and other ingredients as are well known in the ophthalmology art. If the composition is supplied as a ready to inject single dose, then a preservative is typically omitted. The composition can be provided as a frozen liquid or as a lyophilized powder for reconstituting.

Diagnostic materials include gases and dye solutions. For example, a gas such as nitrogen, air, or other inert gas, can be supplied in order to inflate the area and aid in some types of diagnostic procedures; i.e., improving the image in an ophthalmoscope. Similarly, a dye can be injected in order to aid in diagnosing various conditions by providing higher contrast and/or a staining pattern.

The present invention can be used to treat a variety of ocular diseases or conditions including, but not limited to, cystoid macular edema, age-related macular degeneration, diabetic retinopathy, diabetic maculopathy, central retinal artery occlusion, central retinal vein occlusion, branch retinal artery occlusion, branch retinal vein occlusion, retinopathy of prematurity, sickel cell retinopathy, photic retinopathy, radiation retinopathy, retinal detachment, retinitis pigmentosa, macular hole, cataract, and glaucoma as well as accidental or surgically induced trauma to the eye. Suitable therapeutic materials, known for treatment of an ocular disease or condition, especially a retinal disease or condition, can be injected into the sclera in close proximity to the affected site by the present invention to thereby provide effective treatment and enhanced delivery.

For example, one embodiment of the invention relates to treating neovascular diseases of the eye, such as diabetic retinopathy, macular degeneration, and neovascularization of the retina or choroid, by injecting into the sclera, through a location on the exterior surface of the sclera that overlies retinal tissue, an effective neovascularization reducing or preventing amount of an anti-angiogenesis agent. Such agents are described above and are generally well known in the art, including metalloproteinase inhibitors, vascular endothelium growth factor regulating agents, FGF regulating agents, integrin blockers, and protein kinase C inhibitors. The VEGF regulating agents include, without limitation, VEGF, antisense compounds thereof, antibodies thereof, and antibody fragments thereof having anti-angiogenesis activity.

Antioxidants are also a highly useful class of compounds for treating these types of diseases.

With regard to macular degeneration, it is preferred that the insertion site is over the macula or in its immediate vicinity (e.g., more than 45 degrees posterior to the equator). More preferably, the insertion and injection steps provide at least a portion of the intrasclerally injected therapeutic material overlying the macula. The material to be injected can be any macular degeneration treating material. The "macular degeneration treating material" embraces substances that seek to relieve the symptoms of the disease, counteract the cause(s) of the disease or offset the disease by regenerating cells. Specifically contemplated active agents include the above anti-angiogenesis compounds of VEGF, an antisense compound of VEGF, an antibody of VEGF, a fragment of an antibody of VEGF, triplex nucleic acids of VEGF, a receptor blocker for VEGF, ribozymes for VEGF, telomerase, genes encoding for telomerase, and gene therapy vectors including nanoparticles, adeno viral vectors, adeno-associated viruses, retrovirus vectors, picorna viral vectors, liposomes, cationic lipid systems and protein/nucleic acid complexes, as well as antioxidants.

Another embodiment is treating cataracts. Although the disease manifests in the anterior segment, its root cause may lie in the posterior segment. Providing an antioxidant into the posterior segment can prevent or reduce cataracts. Here a preferred antioxidant is a 21-aminosteroid such as a lazaroid.

The following examples serve to illustrate the materials that can be injected by the present invention and should not be considered to limit the scope of the present invention.

Example 1

An injectable therapeutic material containing the PAF antagonist Lexipafant (BB-882) is prepared as follows. In a 250-ml beaker, about 50 g of DI water

is added and heated up to 80-90°C on a hot plate while stirring with a magnetic stir bar. HPMC is dispersed into the hot water and stirred for 15 min. followed by cooling to RT while stirring. 10 g of room temperature water is then added to the polymer and stirred for 10 min. In a separate container, Pluronic F-127 and sorbitol are dissolved in 20 g of DI water. Glycerin is then added to the Pluronic F-127 solution and stirred until dissolve completely. The Pluronic F-127 solution is then added to the polymer suspension and stirred for 10 min. BB-882, dissolved in 1N HCl solution, is then added to the polymer mixture with stirring for 10 min. The pH of the resulting polymer mixture is adjusted to about 7.4 with 2N NaOH, stirred for 10 min., and then brought to 100% with q.s. of DI water. The formulation may be made sterile by heating the formulation to 123°C for 30 minutes and sterile filtering the drug, NaOH, and residual water after heating. The 100 grams of material is summarized in the following table:

COMPOSITION	% (Wt/Wt)
BB-882	1.0
Hydroxylpropyl Methylcellulose, Type 2910, USP	2.5
Sorbitol, USP	1.5
Glycerin, USP	1.0
Pluronic F-127, NF	1.0
Hydrochloric Acid, (1N solution)	5.0
Sodium Hydroxide, NF, 2N for pH adjustment	q.s. to pH 7.4
Purified Water, USP	q.s.

Total weight: 100 grams

Example 2

In a manner similarly to that set forth in Example 1, the following materials can also be made:

COMPOSITION	% (Wt/Wt)
BB-882	0.1
Hydroxylpropyl Methylcellulose, Type 2910, USP	2.5
Sorbitol, USP	1.5
Glycerin, USP	0.2
Edetate Disodium, USP	0.10
Sodium Chloride, USP	0.32
Sodium Hydroxide, NF, 2N for pH adjustment	q.s. to pH 6
Purified Water, USP	q.s.

Total weight: 100 grams

Example 3

In a manner similarly to that set forth in Example 1, the following materials can also be made:

COMPOSITION	% (Wt/Wt)
Diclofenac Na, USP	0.1-1.0
Hydroxylpropyl Methylcellulose, Type 2910, USP	2.5
Mannitol, USP	1.5
Sodium Chloride, USP	0.21
Poloxamer 407, NF	0.05
Boric Acid, USP	0.5
Magnesium Chloride, USP	0.05
Sodium Hydroxide, NF, 2N for pH adjustment	q.s. to pH 6
Purified Water, USP	q.s.

Total weight: 100 grams

Example 4

Lazaroids are known to be potentially useful in treating a variety of ocular ischemic diseases such as glaucoma and diabetic retinopathy. Suitable formulations may be generally formulated as follows. 0.005 grams of the aminosteroid is dissolved into a saline solution formed of 0.9 grams of sodium chloride dissolved in intravenous grade water. The pH is then adjusted to 7.4 with NaOH and the total weight adjusted with

water to 100 grams. The mixture is then sterilized. Alternatively, if the aminosteroid is not a powder, but is a lipid emulsion or in a liposome, it can be dispersed in the saline solution. A suspension can be made by adding hyaluronic acid such as sodium hyaluronate, or other suitable polymer, typically about 1.0 grams and with an increase in the amount of the agent, such as to 0.05 grams. The suspension need only remain sufficiently viscous to allow injection. Suitable aminosteroids include U-74006F, U-74500A, and U-75412A.

Another formulation is to slowly add 10 grams of U-74006F to 950 ml of pure water having 20 millimoles of citric acid under an inert atmosphere and with stirring. Three millimoles of sodium citrate and 8 millimoles of sodium chloride are added with stirring until a clear solution is obtained. The solution can then be sterilized.

Example 5

An injectable therapeutic material containing Batimastat (BB-94) is prepared as follows. In a 250-ml beaker, about 50 g of DI water is added and heated up to 80-90°C on a hot plate while stirring with a magnetic stir bar. HPMC is dispersed into the hot water and stirred for 15 min. followed by cooling to RT while stirring. 10 g of room temperature water is then added to the polymer and stirred for 10 min. In a separate container, Pluronic F-127 and sorbitol are dissolved in 20 g of DI water. Glycerin is then added to the Pluronic F-127 solution and stirred until dissolve completely. The Pluronic F-127 solution is then added to the polymer suspension and stirred for 10 min. BB-94 is then added to the polymer mixture with stirring for 10 min. The pH of the resulting polymer mixture is adjusted to about 6.0 with 2N NaOH, stirred for 10 min., and then brought to 100% with q.s. of DI water. The formulation may be made sterile by heating

the formulation to 123°C for 30 minutes and sterile filtering the drug, NaOH, and residual water after heating. The 100 grams of material is summarized in the following table:

COMPOSITION	% (Wt/Wt)
BB-94	0.3
Hydroxylpropyl Methylcellulose, Type 2910, USP	2.5
Sorbitol, USP	1.5
Glycerin, USP	1.0
Pluronic F-127, NF	1.0
Sodium Hydroxide, NF, 2N for pH adjustment	q.s. to pH 6.0
Purified Water, USP	q.s.

Total weight: 100 grams

Example 6

An injectable Adeno virus vector (AVV) at a titer of 1 to 5 x 10^5 /ml in phosphate buffered saline is prepared as follows. The AVV is prepared by diluting a concentrated solution of virus using phosphate buffered saline, pH 7.0, such that 20 microliters contains a multiplicity of infection of 0.2 to 0.6 virus particles/pigmented retinal epithelial cell.

The invention having been thus described, it will be obvious that the same may be varied in many ways without departing from the scope and spirit of the invention as defined by the following claims.

We claim:

1. A method of intrascleral injection, which comprises:

injecting into the scleral layer of an eye through a location on the exterior surface of the sclera that overlies retinal tissue an effective amount of a therapeutic or diagnostic material.

2. The method according to claim 1, wherein the amount of material injected is at least 0.1 µl.

3. The method according to claim 1, wherein the material comprises a pharmaceutically active agent and an ophthalmically acceptable carrier.

4. The method according to claim 3, wherein said ophthalmically acceptable carrier is water or oil.

5. The method according to claim 3, wherein said pharmaceutically active agent is selected from the group consisting of metalloproteinase inhibitors, vascular endothelium growth factor regulating agents, fibroblast growth factor regulating agents, integrin blockers, protein kinase C inhibitors, endogenous angiogenesis inhibitors, calcium channel blockers, NMDA receptor antagonists, AMPA receptor antagonists, antioxidants, peroxidation inhibitors, apoptosis inhibitors, adenosine or adenosine regulating agents, nitric oxide regulating agents, anti-inflammatory agents, antiviral agents, antibiotics; antitumor agents, anti-cataract agents, anti-glaucoma agents, anesthetics, antibodies and fragments thereof, antisense compounds, ribozymes, and triplex nucleic acids.

6. The method according to claim 3, wherein said material further comprises a biodegradable polymer matrix.

7. The method according to claim 1, wherein said injection is made posteriorly to the equator of the eye.

8. The method according to claim 7, wherein said location on the exterior of the sclera through which said injection is made substantially overlies the macula or its immediate vicinity.

9. The method according to claim 1, which further comprises inserting a cannula into said sclera and carrying out said injecting step by transmitting said material through the cannula into said scleral layer.

10. The method according to claim 9, wherein said cannula is inserted into said scleral layer in a substantially rotational direction.

11. The method according to claim 9, wherein said cannula is inserted into said scleral layer a distance greater than the thickness of the sclera at the location of insertion.

12. The method according to claim 9, wherein said insertion is carried out using a guided injection device.

13. The method according to claim 9, wherein said cannula is inserted at an insertion angle of less than about 60 degrees.

14. The method according to claim 13, wherein said cannula is inserted at an insertion angle of from about 20 to 40 degrees.

15. The method according to claim 9, wherein said cannula is inserted in a fail safe orientation.

16. The method according to claim 9, wherein the material is injected at a rate of from about 0.1 to about 3.0 μl/s.

17. The method according to claim 16, wherein the amount of material injected is within the range of about 3 to about 25 μl.

18. The process according to claim 9, wherein said material is injected into said scleral layer under sufficient force that at least a portion of said material is propelled through said scleral layer.

19. The method according to claim 18, wherein the material is injected at a rate of at least 4 μl/s.

20. The method according to claim 18, wherein said material comprises a colloidal suspension.

21. The method according to claim 20, wherein said colloidal suspension comprises particles ranging in size from 50 to about 150 nanometers.

22. The method according to claim 9, further comprising, making ~~an~~ incision in a conjunctival layer and passing said cannula through said incision, prior to said insertion step into the scleral layer.

23. The method according to claim 1, wherein said material is injected into said scleral layer by needle-less injection.

24. The method according to claim 23, wherein said material is accelerated so as to impact said location on the exterior surface of the sclera at sub-sonic speeds.

25. The method according to claim 23, wherein said material is a liquid or a particulate suspension whose particles are not more than about 40 nanometers.

26. The method according to claim 23, wherein a portion of said material is conveyed into and through said scleral layer.

27. The method according to claim 26, wherein a majority of said material is conveyed into, through and out the interior surface of said scleral layer.

28. The method according to claim 1, further comprising repeating said injecting step through one or more locations on said scleral layer.

29. The method according to claim 1, wherein said eye is suffering from an ocular disease and said material is a therapeutic material effective for treating said disease.

30. The method according to claim 29, wherein said disease is selected from the group consisting of cystoid macular edema, age-related macular degeneration, diabetic

retinopathy, diabetic maculopathy, central retinal artery occlusion, central retinal vein occlusion, branch retinal artery occlusion, branch retinal vein occlusion, retinopathy of prematurity, sickel cell retinopathy, photic retinopathy, radiation retinopathy, retinal detachment, retinitis pigmentosa, macular hole, cataract, and glaucoma.

31. The method according to claim 30, wherein said disease is diabetic retinopathy and said therapeutic material comprises an anti-angiogenesis agent.

32. The method according to claim 30, wherein said disease is glaucoma.

33. A method for treating posterior ocular tissue, which comprises:
forming a depot of a therapeutic material within the sclera of an eye at a location that overlies retinal tissue, wherein said therapeutic material diffuses over time through said sclera and into the underlying tissue or tissues in an effective amount.

34. A method for treating ocular tissue, which comprises:
propelling a diagnostic or therapeutic material through at least a portion of a scleral layer and into at least the underlying choroidal or retinal tissue.

35. The method according to claim 34, which further comprises inserting a cannula through a location on the exterior surface of the sclera that overlies retinal tissue into the scleral layer and injecting said material through said cannula and into said scleral layer with sufficient force that a portion of said material is propelled the remainder of the way through said scleral layer and onto the underlying choroidal or retinal layers.

36. A method for treating macular degeneration, which comprises injecting an effective macular degeneration treating amount of a macular degeneration treating material into the sclera of an eye in need of treatment thereof through a location on the exterior surface of the sclera that overlies retinal tissue.

37. The method according to claim 36, wherein said macular degeneration treating material is selected from the group consisting of antisense compounds of VEGF, antibodies of VEGF, a fragment of an antibody of VEGF, triplex nucleic acids of VEGF, receptor blockers for VEGF, ribozymes for VEGF, telomerase, genes encoding for telomerase, nanoparticles, adeno viral vectors, adeno-associated viruses, retrovirus vectors, picorna viral vectors, liposomes, cationic lipid systems and protein/nucleic acid complexes, and antioxidants.

38. The method according to claim 36, wherein said agent is injected into a portion of the sclera that substantially overlies the macula or its immediate vicinity.

39. A method for treating a condition in the eye involving neovascularization, which comprises injecting into the sclera, through a location on the exterior surface of the sclera that overlies retinal tissue, an effective neovascularization reducing or preventing amount of an anti-angiogenesis agent.

40. The method according to claim 39, wherein said agent is selected from the group consisting of metalloproteinase inhibitors, VEGF regulating agents, FGF regulating agents, integrin blockers, and protein kinase C inhibitors.

41. The method according to claim 39, wherein said condition is diabetic retinopathy.

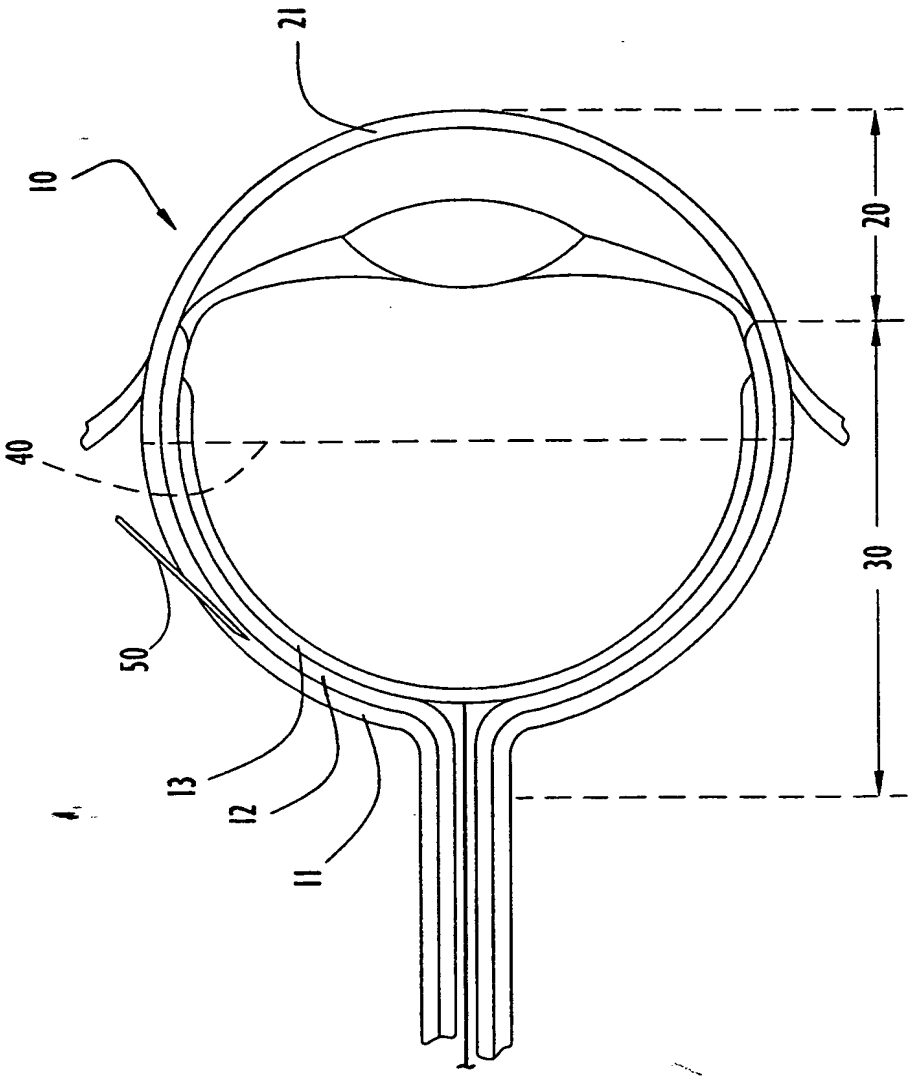


FIG.1

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7]:
A61K 9/00, 38/55, 38/46, 48/00, 39/395, A61P 21/02

A3

(11) International Publication Number: **WO 00/07565**

(43) International Publication Date: 17 February 2000 (17.02.00)

(21) International Application Number: PCT/US99/17543

(22) International Filing Date: 2 August 1999 (02.08.99)

(30) Priority Data:
09/127,920 3 August 1998 (03.08.98) US

(71) Applicant: INSITE VISION, INCORPORATED [US/US]; 965 Atlantic Avenue, Alameda, CA 94588 (US).

(72) Inventors: BOWMAN, Lyle, M.; 5135 Mount Tam Circle, Pleasant, CA 94588 (US). PFEIFFER, James, F.; 2369 Thackery Avenue, Oakland, CA 94611 (US). CLARK, Leslie, A.; 829 #B Lincoln Avenue, Alameda, CA 94501 (US). HECKER, Karl, L.; 400 Hurricane Road, Keene, NH 03431-2161 (US).

(74) Agents: FREED, Joel, M. et al.; Howrey & Simon, 1299 Pennsylvania Avenue, N.W., Box 34, Washington, DC 20004-2402 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
11 May 2000 (11.05.00)

(54) Title: METHODS OF OPHTHALMIC ADMINISTRATION

(57) Abstract

Intrascleral injection of a therapeutic or diagnostic material at a location overlying the retina provides a minimally invasive technique for delivering the agent to the posterior segment of the eye. The procedure also allows for close proximity of the material to the targeted site and can be effectively used to treat conditions associated with the posterior segment of the eye, including macular degeneration, vein occlusion, and diabetic retinopathy. The sclera can be used to hold a depot of the material such as for sustained released or as a conduit for propelling material through whereby the material is delivered immediately to the underlying tissues but without physically penetrating the sclera with an instrument or otherwise unreasonably traumatizing the eye.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 99/17543

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K38/55 A61K38/46 A61K48/00 A61K39/395
A61P21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US VERBEEK A M ET AL: "Recurrent intrascleral cyst after strabismus surgery." retrieved from STN Database accession no. 97024955 XP002130928 abstract & GRAEFES ARCHIVE FOR CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY, (1996 AUG) 234 SUPPL 1 S229-31. , -/--	1-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2000	02/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Ryckebosch, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/17543

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 09838 A (CELTRIX PHARMACEUTICALS, INC.) 4 April 1996 (1996-04-04) page 6, line 20 -page 7, line 31; claims 1,6,15 page 15, line 1 -page 20, line 33 page 22, line 6 - line 8	1-41
Y	WO 92 08406 A (THE UNIVERSITY OF ROCHESTER) 29 May 1992 (1992-05-29) cited in the application page 1, line 5 - line 12; claims 9-11; figure 5 page 3, line 9 - line 15 page 5, line 13 - line 16	1-41
Y	WO 97 41844 A (ALCON LABORATORIES) 13 November 1997 (1997-11-13) page 5, line 4 - line 5; claims page 20, line 10 -page 21, line 15 page 23, line 1 - line 12	1-41

INTERNATIONAL SEARCH REPORT

Internat...al application No.
PCT/US 99/17543

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-41 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/17543

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9609838 A	04-04-1996	AU 3584895 A	19-04-1996
WO 9208406 A	29-05-1992	US 5273530 A	28-12-1993
		AU 9062891 A	11-06-1992
		CA 2096006 A	15-05-1992
		EP 0557445 A	01-09-1993
		JP 6505647 T	30-06-1994
		US 5409457 A	25-04-1995
WO 9741844 A	13-11-1997	AU 2438297 A	26-11-1997

C 24

Device Drug Delivery to the Eye

Collagen Shields, Iontophoresis, and Pumps

MURRAY L. FRIEDBERG, MD, UWE PLEYER, MD, BARTLY J. MONDINO, MD

Abstract: External devices have been used to enhance drug delivery. This article reviews the role of collagen shields, iontophoresis, and pumps used to deliver ophthalmic medications. Collagen shields have been used to deliver drugs and promote corneal epithelial healing. Presoaked collagen shields deliver many drugs to the eye as well as or better than traditional methods such as frequent topical therapy or subconjunctival injection. The efficacy of drug delivery by collagen shields was demonstrated in animal models of graft rejection and bacterial keratitis. Iontophoresis uses an electrical current to carry an ionized drug across tissue. Transcorneal iontophoresis delivers high concentrations of a drug to the anterior segment of the eye. Transscleral iontophoresis bypasses the lens–iris diaphragm and produces adequate vitreous levels. Pumps deliver fluid to the eye for extended periods of time via a tube with its distal opening in the conjunctival sac, corneal stroma, anterior chamber, or vitreous cavity. Clinical acceptance of the collagen shield for drug delivery to the anterior segment is better than iontophoresis or pumps, probably because the collagen shield is simpler and more convenient to use.
Ophthalmology 1991; 98:725–732

Drug delivery to the eye depends on many factors that include concentration of the drug, the contact time at the corneal surface, and the chemical nature of the compound. These variables have been manipulated to enhance the penetration of drugs. For example, fortified topical antibiotics increase the concentration of the drug applied to the eye. Ointments and subconjunctival injections may prolong contact time. Lipophilic compounds more easily cross the lipid-rich epithelial and endothelial cell membranes, whereas hydrophilic compounds more easily cross the corneal stroma. Biphasic compounds which possess both lipid and water solubility penetrate all corneal layers. External devices also have been used to enhance drug delivery. This article reviews the role of collagen shields, iontophoresis, and pumps used to deliver ophthalmic medications.

Originally received: October 28, 1990.
Revision accepted: January 22, 1991.

From the Jules Stein Eye Institute and the Department of Ophthalmology, UCLA School of Medicine, Los Angeles.

Presented at the American Academy of Ophthalmology Annual Meeting, Atlanta, Oct/Nov 1990.

Supported by NEI grant EY04606, the Wasserman Fund, and the Heed Ophthalmic Foundation and the Jack Rubin Memorial Fellowship (Dr. Friedberg). Dr. Pleyer is currently a Fellow of the Deutsche Forschungsgemeinschaft, Germany, at the Jules Stein Eye Institute, Department of Ophthalmology (Pl 150/1).

Reprint requests to Bartly J. Mondino, MD, Jules Stein Eye Institute, UCLA School of Medicine, 200 Stein Plaza, Los Angeles, CA 90024.

COLLAGEN SHIELDS

The collagen shield is shaped like a contact lens. It is packaged in a dehydrated form and is rehydrated before application. Multiple base curves are not needed because the shield conforms to the shape of the cornea. The Bio-Cor collagen shield (Bausch and Lomb, Rochester, NY) has been the most widely studied, and is made of porcine scleral collagen. Variations in collagen cross-linking in-

Table 1. Available Collagen Shield Products

Brand Name	Manufacturer	Collagen Source	Diameter (mm)	Base Curve (mm)	Water Content (%)	Duration (hrs)
Bio-Cor	Bausch and Lomb	Porcine sclera	14.5	9.0	65.7	12, 24, 72
MediLens	Chiron	Bovine corium	16.0	8.8	83.0	24
Collagen Shield	Chiron	Bovine corium	14.0	9.1	80.0	24

duced by ultraviolet irradiation during manufacturing produce shields lasting approximately 12, 24, or 72 hours. The lens is approximately 0.15 to 0.19 mm thick centrally, 14.5 mm in diameter, and has a base curve of 9.0 mm.[1] The water content is 63 to 65.7%, and the oxygen permeability is comparable with a hydroxyethyl–methacrylate (HEMA) lens of similar water content.[1,2] A comparison of this collagen shield to other available lenses is presented in Table 1.

Collagen shields have been used to promote corneal epithelial healing and to deliver drugs. The mechanical properties of the shield protect the corneal epithelium from the action of the eyelids, and the collagen in the lens may have a beneficial effect on healing. In addition, shields lasting up to 72 hours may provide a more convenient method of corneal protection than repeated patching.

Methods for delivering ocular drugs include frequent topical application, subconjunctival injection, or both. Frequent topical application can be labor-intensive and irritating, whereas subconjunctival injections can be painful for the patient and inconvenient for the physician. A device, such as the collagen shield, that delivers therapeutic levels of medication reliably with a minimum number of applications benefits both patients and ophthalmologists. For example, postoperative antibiotics can be delivered by a presoaked collagen shield applied at the completion of cataract or corneal surgery.[3,4] Of the drug delivery devices described in this article, only collagen shields are used in routine clinical practice.

Drug delivery by collagen shields is dependent on absorption and subsequent release of the medication by the shield. When a solution containing a water-soluble drug is used for rehydration, the drug becomes trapped in the interstices of the collagen matrix. Some drugs may undergo reversible binding to collagen.[5,6] As the shield dissolves, it releases the drug. Shields soaked in water-soluble drugs produced corneal and aqueous levels comparable with frequent topical therapy.[6,7] Similar results were obtained for water-insoluble drugs such as cyclosporine.[8] However, this agent had to be incorporated into the shield at the time of manufacturing.

Drug delivery by collagen shields has been studied in three types of experiments. First, in vitro studies measured the absorption of drugs by collagen shields and characterized their release into solution.[6,9,10] Second, corneal and aqueous levels of drugs delivered by collagen shields were compared with conventional therapy in rabbit models.[6–8,10–14] Finally, the therapeutic potential of collagen shields was studied in animal models of graft rejection[15] and keratitis.[9,16]

IN VITRO ABSORPTION AND RELEASE STUDIES

Saturation of the shield occurs rapidly after placement of the lens in a drug-containing solution.[10] The concentration of tobramycin absorbed by the shield was not significantly different when soaked for 10 minutes or 2 hours. However, the amount of medication absorbed was greater for shields soaked in the more concentrated solution. Shields immersed in tobramycin 4% solution absorbed approximately 800 μg of medication,[9,10] and those in a 20% solution absorbed 5525 μg.[10]

The ability of a presoaked collagen shield to release gentamicin and vancomycin individually and in combination was investigated.[6] For gentamicin, the majority of the drug was released within the first 30 minutes of elution. Vancomycin was released more slowly over 6 hours. Vancomycin may have exhibited a sustained release due to reversible binding with collagen. Serum studies showed that 55% of parenterally administered vancomycin binds to serum proteins as opposed to 10% for gentamicin.[17]

CORNEAL PENETRATION STUDIES

Drug delivery to the anterior segment of rabbit eyes using collagen shields has been compared with conventional therapy (Table 2). Extrapolation of data collected in rabbits to humans is limited by differences in tear volume, blink rate and corneal thickness.[18,19] Comparisons of collagen shields with topical therapy, topical therapy in the presence of a therapeutic soft contact lens, and subconjunctival injection have demonstrated that shields provide equal or better drug delivery to the anterior segment. Collagen shields have been used to deliver antibacterial, antifungal, anti-inflammatory, immunosuppressive, and anticoagulant agents to the eye.

The prolonged exposure time of medication to the cornea provided by a presoaked shield may produce higher tissue levels than a single drop that is rapidly carried away by the tears. Hwang et al[7] demonstrated that a collagen shield presoaked in dexamethasone alcohol provided significantly higher corneal and aqueous levels and greater cumulative drug delivery than a single drop of the drug. A presoaked collagen shield was placed on the right eye of a rabbit, and a single drop of 0.1% dexamethasone was instilled into the left eye. The eyelids were taped to prevent loss of the collagen shield. Animals were killed at intervals over the 6-hour experiment. Both the collagen shield and the single 50-μl drop carried 50 μg of dexamethasone. However, a 50-μl drop may be too large for the conjunctival sac resulting in loss of medication by overflow. Peak

Table 2. Studies of Collagen Shield Drug Delivery

Reference	Drug	Compared with CS	Assay Site	Overall Result
Phinney et al[6] 1988	Gentamicin	Loading dose + frequent drops	Tears Cornea Aqueous	CS comparable at all sites
Phinney et al[6] 1988	Vancomycin	Loading dose + frequent drops	Tears Cornea Aqueous	CS comparable at all sites
O'Brien et al[11] 1988	Tobramycin	Soft contact lens	Aqueous	CS superior
Unterman et al[10] 1988	Tobramycin	Subconjunctival injection	Cornea Aqueous	CS comparable at both sites
Hwang et al[7] 1989	Dexamethasone	Single drop	Cornea Aqueous Iris Vitreous	CS superior at all sites
Hwang et al[7] 1989	Dexamethasone	Frequent drops	Cornea Aqueous Iris Vitreous	CS superior at all sites
Hwang et al[7] 1989	Dexamethasone	CS + frequent drops versus frequent drops	Cornea Aqueous Iris Vitreous	CS superior at all sites
Sawusch et al[13] 1989	Prednisolone	Single drop	Cornea Aqueous	CS superior at both sites
Schwartz et al[12] 1990	Amphotericin B	Frequent drops	Cornea Aqueous	CS comparable at both sites
Reidy et al[8] 1990	Cyclosporin A	Frequent drops	Cornea Aqueous	CS superior CS comparable
Murray et al[14] 1990	Heparin	Subconjunctival injection	Aqueous	CS superior

CS = collagen shield.

tissue levels occurred within 1 hour in both groups. Similar results for prednisolone acetate were found in studies performed by Sawusch et al.[13]

Hwang et al[7] also used a collagen shield as a reservoir for hourly topical therapy. Higher corneal and aqueous levels of dexamethasone alcohol were obtained when hourly topical therapy was administered to eyes wearing a presoaked collagen shield than to those without a shield.

Topical tobramycin was applied to rabbit corneas with either a presoaked 12-hour collagen shield or a low water content hydrogel contact lens in place.[11] Aqueous levels were significantly higher in the shield-treated group. A therapeutic contact lens has been shown to absorb drugs and subsequently release the medication to the tear film.[20] Thicker, higher water content lenses delivered more drug than thinner, lower water content lenses. Hydroxyethylmethacrylate lenses release the drug rapidly, and prolonged delivery does not occur. Hydrogel lenses can become tight and get "stuck on."

Subconjunctival therapy has been used clinically to administer depots of corticosteroids and antibiotics to obtain a prolonged delivery of drug. The medication may slowly leak out of the conjunctival needle hole, providing a continuous flow of drug to the corneal surface, or enter the eye by direct penetration through the sclera. The latter route circumvents the problem of crossing the corneal epithelium. Corneal levels of tobramycin in eyes treated with presoaked shields were greater than those receiving a single subconjunctival injection.[10] Aqueous levels were comparable.

Cyclosporin A (CsA) is poorly soluble in water. Many vehicles have been used to enhance delivery including vegetable oils. Corneal and aqueous levels of CsA were studied by Reidy et al[8] using shields with CsA incorporated into them at the time of manufacturing. Each shield contained 4 mg of CsA, which was the maximum amount that could be incorporated. Controls were treated with topical CsA in olive oil, making an equivalent cumulative dose available to the eye. Corneal and aqueous levels for the shield-treated group were greater than or equal to the control group at all time intervals studied. Levels in aqueous were in the range considered to be adequate to suppress graft rejection[21] based on the blood levels needed to suppress rejection in organ transplants.[22]

The studies described above used corneas with intact epithelium. Phinney et al[6] studied the delivery of gentamicin and vancomycin by collagen shields in corneas with central epithelial defects to simulate corneas with central bacterial ulcers. Since both drugs are polar hydrophobic compounds, removal of the epithelial barrier should increase penetration. Peak levels of gentamicin in the cornea occurred at 30 minutes corresponding to the rapid release of gentamicin from collagen shields demonstrated by in vitro elution studies. Nearly 95% of the drug was released

Table 3. Studies of Collagen Shield Drug Delivery in Rabbit Models of Disease

Reference	Experimental Model	Drug	Compared with CS	Result
Chen et al[15] 1990	High-risk keratoplasty	Cyclosporin A	Drops	Superior preventive effect on graft rejection
			Drops	Superior therapeutic effect on graft rejection
Hobden et al[9] 1988	*Pseudomonas keratitis*	Tobramycin	Frequent drops	Comparable antimicrobial effect
			CS + delayed drops versus second CS	Comparable antimicrobial effect
Sawusch et al[16] 1988	*Pseudomonas keratitis*	Tobramycin	CS + frequent drops versus frequent drops	Enhanced antimicrobial effect with CS

CS = collagen shield.

by 30 minutes. Vancomycin was released more slowly with peak corneal levels occurring at 3 hours, which also was consistent with in vitro elution data. When gentamicin and vancomycin were combined, similar kinetics were found. For tears, cornea, and aqueous, levels delivered by presoaked shields were superior or comparable with hourly topical therapy. At 6 hours, both regimens demonstrated gentamicin levels higher than the therapeutic range often used to define an organism's sensitivity to this antibiotic.

In addition to antibiotics, the antifungal, amphotericin B, has been evaluated.[12] In a rabbit model with central corneal epithelial defects, shields soaked in amphotericin B were compared with hourly topical therapy. Corneal levels for the shield-treated group were comparable with the drop-treated group.

Postoperative anterior chamber fibrin exudation can complicate vitrectomy. The ability of heparin to inhibit aqueous fibrin activity was studied.[14] Delivery of heparin by a presoaked collagen shield was compared with subconjunctival injection. Maximum aqueous heparin activity occurred at 30 minutes for the shield-treated group, corresponding closely to the peak corneal and aqueous heparin levels measured in penetration studies. Subconjunctival delivery of heparin was not associated with significant aqueous heparin activity.

DRUG DELIVERY IN ANIMAL MODELS OF DISEASE

The therapeutic potential of corneal shields has been studied in animal models of disease (Table 3). Rabbit corneas injected intrastromally with *Pseudomonas aeruginosa* were treated with tobramycin.[9] Corneal tissue was removed at predetermined intervals, and homogenates were evaluated quantitatively for viable organisms. Bacterial counts were performed by a masked observer. Shields rehydrated in 4% tobramycin were as effective in reducing the number of bacteria as 4% drops applied every 30 minutes in a 4-hour trial. There was no significant difference in the number of viable organisms remaining in corneas treated with a collagen shield presoaked in distilled water and eyes receiving no treatment. Animals receiving therapy with a collagen shield were sedated and their eyelids were taped to prevent loss of the shield. It is not known whether sedation and eyelid closure would influence the results of the experiment.

In a second study,[9] addition of topical tobramycin to collagen shields in situ was as effective as replacing the shield with a second shield rehydrated in tobramycin. This study also demonstrated the greater efficacy of shields compared with frequent drop therapy. The bacterial eradication produced by half-hourly drop therapy totaling 18 mg of tobramycin available to the eye was no greater than that obtained with a shield containing 800 μg of drug.

Chen et al[15] compared collagen shields impregnated with CsA with topical CsA in olive oil in a high-risk rabbit model of corneal allograft rejection. In the first experiment, CsA was given immediately after transplant to prevent graft rejection. Ninety percent of the shield-treated group remained clear for the 120-day trial. Only 20% of the drop-treated grafts survived. In the second experiment, CsA was withheld until early graft rejection was present. Mean survival time of grafts subsequently treated with CsA-impregnated shields was nearly 250% greater than grafts treated topically with CsA. Results of histopathologic examination of the failed grafts demonstrated a less intense rejection reaction with fewer T lymphocytes and HLA class II expressing cells in the shield-treated group.

COMBINING DRUGS IN COLLAGEN SHIELDS

An antibiotic and a corticosteroid are often injected in the subconjunctival space after routine intraocular surgery. However, subconjunctival injections can be painful at the conclusion of surgery, even after an adequate retrobulbar anesthetic injection. Collagen shields have been used to deliver drugs after anterior segment surgery to avoid subconjunctival injections.[3,4] A recent clinical alert by the American Academy of Ophthalmology (Clinical Alert 11/1, AAO, November 9, 1990) reported patients who had epithelial sloughing and corneal edema after the application of a collagen shield previously soaked in both Solumedrol (40 mg/ml methylprednisolone sodium succinate for injection) and Garamycin (40 mg/ml gentamicin sulfate injection). In vitro mixing of the two drugs produced an aggregate. It was unclear whether the aggregate damaged the cornea by causing chemical toxicity or

Table 4. A Comparison of Iontophoresis to Other Routes of Drug Delivery in Rabbits

Reference	Drug	Site of Delivery	Lens Status	Compared with IP	Assay Site	Overall Result
von Sallmann[46] 1942	Sulfadiazine	Transcorneal	Phakic	Sulfadiazine eye bath	Cornea	IP superior at all sites
					Aqueous	
					Iris	
					Vitreous	
Witzel et al[30] 1956	Tetracyclines	Transcorneal	Phakic	Subconjunctival injection	Aqueous	IP comparable
	Chloramphenicol					IP inferior
	Penicillin G					IP inferior
	Streptomycin					IP superior
	Neomycin					IP superior
	Erythromycin					IP inferior
Fishman et al[33] 1984	Gentamicin	Transcorneal	Aphakic	Gentamicin eye bath	Cornea	IP superior at both
					Aqueous	sites
Hughes and Maurice[28] 1984	Gentamicin	Transcorneal	Phakic	Frequent drops	Aqueous	IP superior
Choi and Lee[36] 1988	Vancomycin	Transscleral	Phakic	Subconjunctival injection	Aqueous	IP inferior
					Vitreous	IP superior
		Transcorneal			Cornea	IP superior
					Aqueous	IP superior
Grossman and Lee[34] 1989	Ketoconazole	Transscleral	Phakic	Subconjunctival injection	Aqueous	IP superior
					Vitreous	IP comparable
		Transcorneal			Cornea	IP superior
					Aqueous	IP comparable

IP = iontophoresis.

by mechanical abrasion. If subconjunctival injections are to be replaced by collagen shields, a compatible combination of corticosteroid and antibiotic must be used. Also, the additional cost of collagen shields must be considered when they are used for postoperative drug delivery.

Two antibiotics are commonly used in combination in the initial treatment of corneal ulcers for broad-spectrum coverage. Phinney et al[6] studied collagen shield delivery of gentamicin and vancomycin to the cornea and aqueous of rabbits. Combining both drugs in a collagen shield did not decrease delivery of either drug when compared with delivery of each drug separately, and therefore should provide broad-spectrum antibiotic coverage to the cornea. On the other hand, combining gentamicin and cefazolin in solution resulted in precipitation, so that this combination could not be incorporated into a collagen shield.

Although certain combinations of drugs may be successfully absorbed and delivered by collagen shields, the drugs may not be compatible pharmacologically. The combination of an aminoglycoside such as gentamicin and a penicillin such as mezlocillin should be avoided because of inactivation of the aminoglycoside by the penicillin.[17]

IONTOPHORESIS

Iontophoresis uses an electric current to carry an ionized drug across tissue. This is especially useful for polar fat-insoluble drugs such as gentamicin which cross cell membranes poorly. Ocular delivery requires currents of only 1 to 2 mA. Iontophoresis has been used to test for cystic fibrosis by delivering pilocarpine transcutaneously,[23] to induce herpes simplex virus type 1 shedding by delivering high concentrations of epinephrine to latently infected rabbit eyes,[24,25] to treat herpes orolabialis with vidarabine monophosphate,[26] and to administer local anesthetics to the outer ear canal and tympanic membrane.[27] Iontophoresis has not gained wide acceptance because of unfamiliar equipment and electrical requirements available.

Corneal iontophoresis delivers high concentrations of a drug to the anterior segment of the eye. In phakic animals, the lens–iris diaphragm limits penetration of a drug into the vitreous, whereas higher vitreous levels are achieved in aphakic animals. The lens–iris barrier can be bypassed by applying the current through the pars plana (transscleral iontophoresis), producing significant vitreous levels in phakic rabbits (Table 4).

CORNEAL IONTOPHORESIS

In the technique of corneal iontophoresis,[28] an eyecup which is filled with medication is placed over the cornea. A platinum electrode is positioned in contact with the solution. To drive electropositive drugs (cations) from the eyecup solution into the cornea, the positive pole of the circuit (anode) is connected to the eyecup electrode. The negative pole (cathode), which is the indifferent or ground electrode, is taped to the neck or ear of an animal or held in the hand of a human. For example, positively charged gentamicin will move from the positive pole in contact with the eyecup solution toward the negatively charged

ocular interior. If a system of similar polarity is used to deliver sodium sulfathiazole, the positively charged sodium ions would be driven into the eye and not the negatively charged sulfathiazole ions. For proper delivery of a negatively charged ion, the negative pole must be connected to the eyecup.

Hughes and Maurice[28] compared iontophoresis of fluorescein and gentamicin with simple immersion of the cornea in these solutions without current. Penetration into the anterior chamber after iontophoresis was increased by 120 and 200 times, respectively. In another study, iontophoresis of vidarabine monophosphate was compared with topical therapy.[29] Levels measured in cornea and aqueous were 4.8 to 17.5 times greater in the iontophoresis-treated eyes. Witzel et al[30] studied iontophoresis of antibiotics. Instead of an eyecup, the electrode was placed in contact with a cotton pad soaked with the drug and placed over closed eyelids. Compared with subconjunctival injection, iontophoresis delivered superior or equivalent aqueous levels of streptomycin and neomycin.

Rootman et al[31] investigated the ability of different tobramycin regimens to eradicate *Pseudomonas* injected into the corneal stroma of rabbits. This study demonstrated that two iontophoresis treatments separated by 5 hours was as effective as half-hourly fortified topical drops in reducing the bacterial counts of infected corneas. In addition, iontophoresis delivered higher corneal tobramycin levels than frequent fortified topical therapy.

In phakic eyes, significant vitreous levels of antibiotic could not be achieved by corneal iontophoresis.[32] However, Fishman was able to demonstrate significant vitreous levels of gentamicin in aphakic rabbit eyes.[33] This situation is clinically relevant since many cases of endophthalmitis occur after cataract surgery.

Although damage associated with corneal iontophoresis has been minimal, persistent corneal opacities were reported with ketoconazole.[34] However, these opacities were thought to have been caused by alkaline chemical injury to the cornea.[35] In a study by Choi and Lee,[36] corneal endothelial cell counts obtained 4 days after corneal iontophoresis of vancomycin were decreased 8.8%.

TRANSSCLERAL IONTOPHORESIS

Briefly, the drug solution is contained in a narrow tube within an eyecup held to the conjunctiva by suction. The tube is placed over the pars plana to avoid the retina. Maximum drug transport occurs when the diameter of the probe tip is 1 mm or less.[37] This technique circumvents the lens–iris diaphragm which limits penetration to the vitreous during corneal iontophoresis.

Barza et al[38] measured vitreal levels of cefazolin, gentamicin, and ticarcillin delivered by transscleral iontophoresis at various amperages and durations. Levels were nearly twice as high with 2.0 mA for 5 minutes than 1 mA for 10 minutes, suggesting that intensity of the current was more important than duration of treatment. Drug delivery was not significantly changed by a fourfold decrease in the concentration of the iontophoresis solution. However, to maintain the same amperage in the more dilute solution, the voltage level had to be increased. Drug delivery did not correlate with the charge on the molecule. Levels for ticarcillin and cefazolin were similar although they carry a different number of charges. Gentamicin levels were nearly twice that of ticarcillin although both carry two (but opposite) charges.

Ketoconazole is an imidazole antifungal agent. Achieving adequate intraocular concentrations by traditional modes of drug delivery has been a problem. Transscleral iontophoresis of ketoconazole produced higher aqueous levels than transcorneal iontophoresis or subconjunctival injection.[34] None of these methods produced significant vitreous levels.

Because of the potential risks and the need to bring the patient to the operating room, physicians may be reluctant to perform repeat intravitreal antibiotic injections. In a rabbit model of *Pseudomonas* endophthalmitis, intravitreal injection of gentamicin alone was compared with intravitreal injection supplemented with daily transscleral iontophoresis of gentamicin for 2 consecutive days.[39] Iontophoresis-treated eyes had greater reduction in bacterial counts, greater percentage of eyes considered sterilized (<10 cfu/ml), and greater concentration of gentamicin in the vitreous at the time the animal was killed.

Histopathologic changes after transscleral iontophoresis were confined to a 2-mm diameter area surrounding the contact site of the iontophoresis solution on the globe.[39] Abnormalities including hemorrhagic necrosis, edema, and infiltration of polymorphonuclear cells were detected in the retina, choroid, and ciliary body. Indirect ophthalmoscopy in monkeys, 2 months after receiving three transscleral gentamicin iontophoresis treatments, demonstrated 1- to 2.5-mm burns at the pars plana.[40] No electroretinographic abnormalities were detected. The damage produced by transscleral iontophoresis is probably no greater than that produced by a needle inserted through the pars plana during vitreous culture.

PUMPS

Pumps are small devices that deliver fluid to the eye via a tube for extended periods of time. They are usually implanted in the subcutaneous space and may not require an external power source or battery. The tube is passed from the pump to the eye through a subcutaneous tunnel. The distal end of the tube can be located in the conjunctival sac, corneal stroma, anterior chamber, or vitreous cavity.

In two chamber pumps such as the Infusaid (Metal Bellows Corporation, Sharon, MA), one chamber contains the drug in aqueous solution and the other a charging solution. The charging fluid is a fluorocarbon in liquid–gas equilibrium that expands at body temperature driving the aqueous fluid at a constant flow rate. Rehkopf et al[41] used a two-chamber pump which was implanted in a subcutaneous pouch in the lumbar region to deliver fluorescein dye to the tear film of rabbits. A silicone tube connected the pump to a polytetrafluoroethylene tube located

in the superior fornix. A continuous flow was maintained until the experiment was terminated at 6 weeks. The pump could be refilled transcutaneously. No extrusion or infections were noted.

The Alzet osmotic minipump (Alzet Pharmaceuticals, Palo Alto, CA) is driven by osmotic imbibition of extracellular water. The pump will deliver fluid for approximately 7 days. In a model of *Staphylococcus aureus* endophthalmitis in rabbits, treatment with a single intravitreal gentamicin injection was compared with gentamicin delivered by an Alzet osmotic minipump implanted subcutaneously.[42] Five days after initiation of therapy, 80% of eyes treated with a single intravitreal injection were culture-negative. Only 40% of eyes treated with the minipump were culture-negative. Both groups were treated with 100 μg of gentamicin. The drug was delivered as a bolus by a single intravitreal injection, whereas the minipump delivered the antibiotic over 5 days, giving lower concentrations at any point in time.

Cyclosporin delivery by an osmotic minipump was studied in a rabbit model of corneal allograft rejection.[43] Osmotic minipumps were used to deliver cyclosporine in olive oil to corneal grafts of experimental rabbits and olive oil alone to corneal grafts of control rabbits. An allograft reaction developed in only 43% of the cyclosporine-treated grafts, whereas all corneal grafts receiving olive oil without cyclosporine rejected.

Eliason and Maurice[44] used an osmotic minipump to deliver fluid through a polyethylene tube into the corneal stroma. Corneal edema was noted within 1 to 2 mm of the tube opening, although the tube was well tolerated and maintained patency. This system could be used to determine the long-term effects on the cornea of various compounds which cannot cross the corneal epithelium.

Miki et al[45] demonstrated the long-term tolerance of the eye to an intraocular cannula that could be attached to a pump. The cannula entered the eye through the pars plana and then opened into the vitreous. In eyes with the cannula in situ for up to 18 months, no retinal detachment, epiretinal membranes, or vitreous traction bands were noted. Minimal cellular proliferation and inflammation were noted around the cannulae.

Pumps have not gained acceptance in clinical ophthalmology. The surgical procedure required for their insertion and potential problems with infection and extrusion limit their usefulness. However, pumps could prove useful in research. The long-term tolerance of ocular structures to medications or toxins could be studied with a continuous flow of drug into the corneal stroma, anterior chamber, or vitreous cavity. Tubes emptying into the conjunctival sac could be used for similar studies or for bathing a corneal ulcer with a continuous flow of antibiotics.

REFERENCES

1. Weissman BA, Lee DA. Oxygen transmissibility, thickness, and water content of three types of collagen shields. Arch Ophthalmol 1988; 106:1706–8.
2. Weissman BA, Brennan NA, Lee DA, Fatt I. Oxygen permeability of collagen shields. Invest Ophthalmol Vis Sci 1990; 31:334–8.
3. Aquavella JV, Ruffini JJ, LoCascio JA. Use of collagen shields as a surgical adjunct. J Cataract Refract Surg 1988; 14:492–5.
4. Poland DE, Kaufman HE. Clinical uses of collagen shields. J Cataract Refract Surg 1988; 14:489–91.
5. Aquavella JV. Collagen shield delivery of amphotericin B [letter]. Am J Ophthalmol (in press)
6. Phinney RB, Schwartz SD, Lee DA, Mondino BJ. Collagen-shield delivery of gentamicin and vancomycin. Arch Ophthalmol 1988; 106: 1599–1604.
7. Hwang DG, Stern WH, Hwang PH, MacGowan-Smith LA. Collagen shield enhancement of topical dexamethasone penetration. Arch Ophthalmol 1989; 107:1375–80.
8. Reidy JJ, Gebhardt BM, Kaufman HE. The collagen shield. A new vehicle for delivery of cyclosporin A to the eye. Cornea 1990; 9: 196–9.
9. Hobden JA, Reidy JJ, O'Callaghan RJ, et al. Treatment of experimental *Pseudomonas* keratitis using collagen shields containing tobramycin. Arch Ophthalmol 1988; 106:1605–7.
10. Unterman SR, Rootman DS, Hill JM, et al. Collagen shield drug delivery: therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humor. J Cataract Refract Surg 1988; 14:500–4.
11. O'Brien TP, Sawusch MR, Dick JD, et al. Use of collagen corneal shields versus soft contact lenses to enhance penetration of topical tobramycin. J Cataract Refract Surg 1988; 14:505–7.
12. Schwartz SD, Harrison SA, Engstrom RE Jr, et al. Collagen shield delivery of Amphotericin B. Am J Ophthalmol 1990; 109:701–4.
13. Sawusch MR, O'Brien TP, Updegraff SA. Collagen corneal shields enhance penetration of topical prednisolone acetate. J Cataract Refract Surg 1989; 15:625–8.
14. Murray TG, Stern WH, Chin DH, MacGowan-Smith EA. Collagen shield heparin delivery for prevention of postoperative fibrin. Arch Ophthalmol 1990; 108:104–6.
15. Chen YF, Gebhardt BM, Reidy JJ, Kaufman HE. Cyclosporine-containing collagen shields suppress corneal allograft rejection. Am J Ophthalmol 1990; 109:132–7.
16. Sawusch MR, O'Brien TP, Dick JD, Gottsch JD. Use of collagen corneal shields in the treatment of bacterial keratitis. Am J Ophthalmol 1988; 106:279–81.
17. Sande MA, Mandell GL. Antimicrobial agents. In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. Goodman and Gilman's Pharmacological Basis of Therapeutics, 7th ed. New York: Macmillan, 1985; 1154–92.
18. Chral SS, Patton TF, Mehta A, Robinson JR. Lacrimal and instilled fluid dynamics in rabbit eyes. J Pharm Sci 1973; 62:1112–21.
19. Prince JH. The Rabbit in Eye Research. Springfield: Charles C. Thomas, 1964; 130.
20. Waltman SR, Kaufman HE. Use of hydrophilic contact lenses to increase ocular penetration of topical drugs. Invest Ophthalmol 1970; 9:250–5.
21. The Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplant: analysis at three years. N Engl J Med 1986; 314:1219–25.
22. Kahan BD, van Buren CT, Flechner SM, et al. Clinical and experimental studies with cyclosporine in renal transplantation. Surgery 1985; 97: 125–40.
23. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 1959; 23:545–9.
24. Hill JM, Shimomura Y, Kwon BS, Gangarosa LP. Iontophoresis of epinephrine isomers to rabbit eyes induced HSV-1 ocular shedding. Invest Ophthalmol Vis Sci 1985; 26:1299–303.
25. Hill JM, Kwon BS, Shimomura Y, et al. Herpes simplex virus recovery in neural tissues after ocular HSV shedding induced by epinephrine iontophoresis in the rabbit cornea. Invest Ophthalmol Vis Sci 1983; 24:243–7.

26. Gangarosa LP Sr, Hill JM, Thompson BL, et al. Iontophoresis of vidarabine monophosphate for herpes orolabialis. J Infect Dis 1986; 154:930–4.
27. Bridges MQM, Keene M, Graham JM. A device for iontophoretic anesthesia of the tympanic membrane. J Med Technol 1982; 6:62–4.
28. Hughes L, Maurice DM. A fresh look at iontophoresis. Arch Ophthalmol 1984; 102:1825–9.
29. Hill JM, Park N-H, Gangarosa LP, et al. Iontophoresis of vidarabine monophosphate into rabbit eyes. Invest Ophthalmol Vis Sci 1978; 17: 473–6.
30. Witzel SH, Fielding IZ, Ormsby HL. Ocular penetration of antibiotics by iontophoresis. Am J Ophthalmol 1956; 42 (no. 2, pt. 2):89–95.
31. Rootman DS, Hobden JA, Jantzen JA, et al. Iontophoresis of tobramycin for the treatment of experimental *Pseudomonas* keratitis in the rabbit. Arch Ophthalmol 1988; 106:262–5.
32. von Sallmann L. Penetration of penicillin into the eye: further studies. Arch Ophthalmol 1945; 34:195–201.
33. Fishman PH, Jay WM, Rissing JP, et al. Iontophoresis of gentamicin into aphakic eyes: sustained vitreal levels. Invest Ophthalmol Vis Sci 1984; 25:343–5.
34. Grossman R, Lee DA. Transscleral and transcorneal iontophoresis of ketoconazole in the rabbit eye. Ophthalmology 1989; 96:724–9.
35. Grossman R, Lee DA. Advice on iontophoresis [letter]. Ophthalmology 1989; 96:1681–2.
36. Choi TB, Lee DA. Transscleral and transcorneal iontophoresis of vancomycin in rabbit eyes. J Ocul Pharmacol 1988; 4:153–64.
37. Maurice DM. Iontophoresis of fluorescein into the posterior segment of the rabbit eye. Ophthalmology 1986; 93:128–32.
38. Barza M, Peckman C, Baum J. Transscleral iontophoresis of cefazolin, ticarcillin, and gentamicin in the rabbit. Ophthalmology 1986; 93: 133–9.
39. Barza M, Peckman C, Baum J. Transscleral iontophoresis as an adjunctive treatment for experimental endophthalmitis. Arch Ophthalmol 1987; 105:1418–20.
40. Barza M, Peckman C, Baum J. Transscleral iontophoresis of gentamicin in monkeys. Invest Ophthalmol Vis Sci 1987; 28:1033–6.
41. Rehkopf PG, Mondino BJ, Brown SI, Goldberg DB. A long-term implantable aqueous delivery system for the external rabbit eye. Invest Ophthalmol Vis Sci 1980; 19:428–30.
42. Michelson JB, Nozik RA. Experimental endophthalmitis treated with an implantable osmotic minipump. Arch Ophthalmol 1979; 97: 1345–6.
43. Roussel TJ, Osato MS, Wilhelmus KR. Cyclosporine and experimental corneal transplantation. Transplant Proc 1983; 15:3081–3.
44. Eliason JA, Maurice DM. An ocular perfusion system. Invest Ophthalmol Vis Sci 1980; 19:102–5.
45. Miki K, Patterson R, Ryan SJ. Intraocular cannula for continuous, chronic drug delivery: histopathologic observations and function. Arch Ophthalmol 1985; 103:712–7.
46. von Sallmann L. Sulfadiazine iontophoresis in pyocyaneus infection of rabbit cornea. Am J Ophthalmol 1942; 25:1292–300.

C25

The MAI Hydrophilic Implant for Scleral Buckling: A Review

Patrick C. Ho, M.D.
Ildefonso M. Chan, M.D.
Miguel F. Refojo, D. Sc.
Felipe I. Tolentino, M.D.

SUMMARY

MAI, a new hydrophilic implant designed by Refojo for scleral buckling, is soft and elastic (thus preventing erosion) and offers versatility in interconvertible degrees of softness. MAI has no dead spaces. Its ability to absorb and gradually release antibiotics and to stimulate the production of a fibrous capsule around the implant increases safety and minimizes morbidity associated with the scleral buckling operation. This review summarizes the works published to date on MAI.

Scleral buckling often is a necessary, efficacious procedure for closing retinal breaks in the surgical repair of rhegmatogenous retinal detachment.[1] The buckling material, however, which is usually placed over tissue injured by cryotherapy or diathermy, may hinder repair of the tissue injury by impeding the free flow of tissue fluid.[2] Silicone sponge, one commonly used buckling material, is not a true sponge but a silicone foam rubber with cavities, and macropores on the cut surfaces. The intercellular walls are easily ruptured by heat sterilization, squeezing, and stretching,[3] resulting in interconnecting spaces within the sponge. These pores and spaces absorb body fluid and may harbor organisms. Reported infection rates after scleral buckling with silicone sponges have ranged from 2.7% to 18.0%.[4,5] Extrusion is also a problem. With solid silicone rubber, the infection rate is lower, between 0.2% and 1.4%,[6,7] but pressure necrosis, erosion, and extrusion can occur. Recently, Refojo synthesized MAI, a material for scleral buckling, which seems to possess the ideal features of an implant material.[8]

From the Eye Research Institute of Retina Foundation, and Retina Associates, Boston, Massachusetts.

Supported by grant EY00327 from the National Eye Institute, Bethesda, Maryland.

Requests for reprints should be addressed to Patrick C. Ho, M.D., Eye Research Institute, 20 Staniford Street, Boston, Massachusetts 02114.

CHEMICAL AND PHYSICAL PROPERTIES

The monomers constituting MAI are 2-hydroxyethyl acrylate, methyl acrylate, and ethylene diacrylate. The polymer (MAI) is copoly(methyl acrylate-2-hydroxyethyl acrylate) cross-linked with ethylene diacrylate. The material is soft, elastic, nonabsorbable, hydrophilic but insoluble, microporous, nonelectrostatic, has smooth surfaces, and can be sterilized by heat.

The hardness of solid silicone rubber is 30 and the hardness of silicone sponge is 6, as measured by the Shore Durometer type A-2. MAI, when dry (xerogel), is transparent and pliable, and has a hardness of 31. At equilibrium (i.e., the highest hydration reached in water or physiological saline), MAI is about 16% water. It becomes white, opaque, and softer, with a hardness of 15. Higher levels of hydration can be obtained by swelling the material in ethanol solution and then placing it in water or isotonic saline which elutes the ethanol. MAI changes from translucent to opaque as water replaces ethanol, and any traces of ethanol remaining in the material are insufficient to harm surrounding tissues. At maximum hydration, reached after preswelling in 70% ethanol, MAI has a hardness of 6 (Figure 1).

SWELLING HYSTERESIS

The swelling hysteresis shows the implant's versatility. By changing the ethanol-water concentration used in pre-swelling, we can alter the degree of implant hydration, swelling, and softness to meet specific needs.

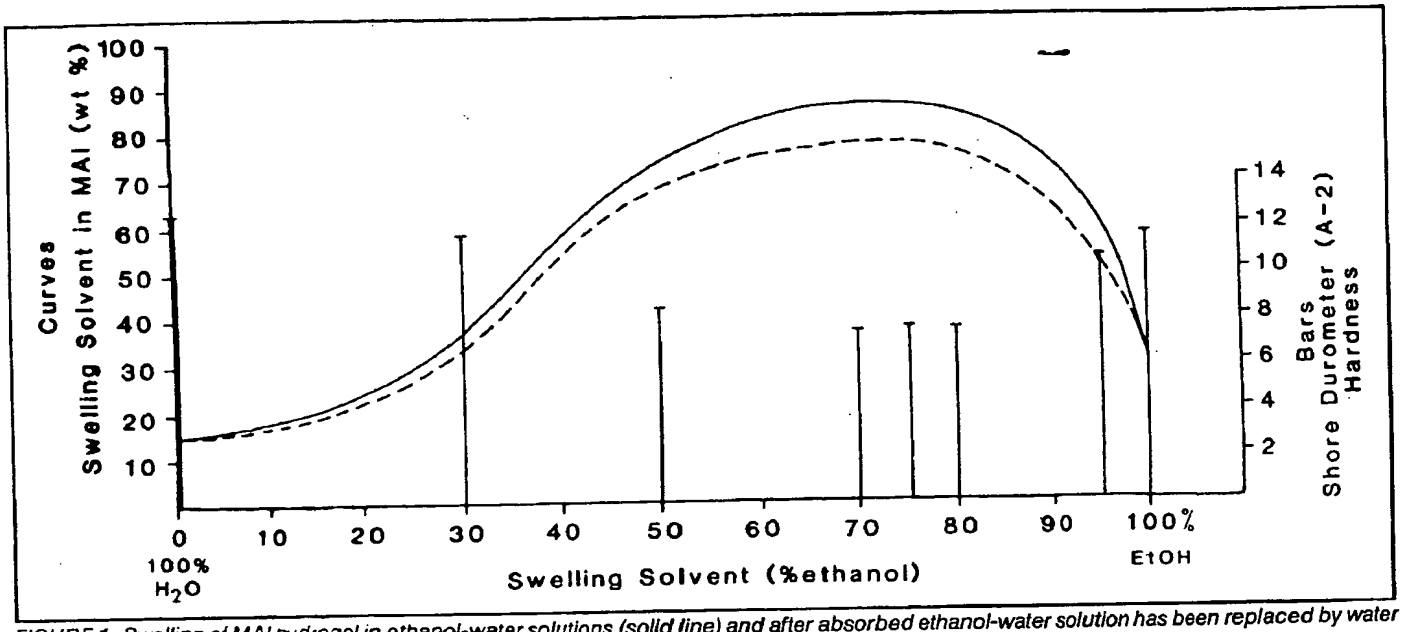


FIGURE 1: Swelling of MAI hydrogel in ethanol-water solutions (solid line) and after absorbed ethanol-water solution has been replaced by water alone (broken line). The vertical lines represent the Shore Durometer hardness of the hydrogel swollen in water.

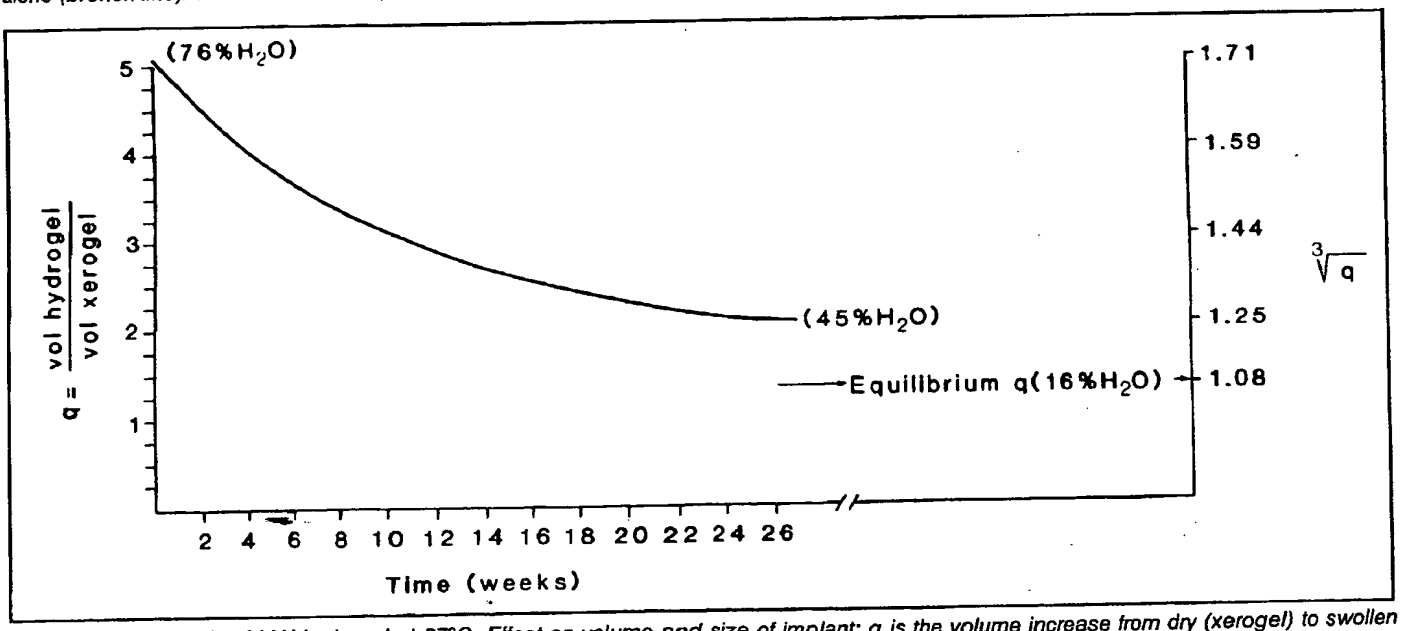


FIGURE 2: Hysteresis of MAI hydrogel at 37°C. Effect on volume and size of implant: q is the volume increase from dry (xerogel) to swollen (hydrogel), i.e., at 76% hydration the volume of the hydrogel is five times its dry volume, and it is about 1.7 times thicker and longer. After 16 weeks an implant will shrink to about half its original volume. Reprinted from J Biomed Mater Res *1981; 15:503.*

When maximally hydrated after being swollen in 70% ethanol, the MAI is about 76% water, and is five times its original dry volume. At 45% water, its volume doubles, and at 16% water, it is 1.2 times its original dry volume (Figures 2 & 3).[9] Thus, if implanted in its dry state, the implant may absorb body fluid over time, and a greater buckling effect may result. When it is implanted at equilibrium hydration (16% water), constant buckle size is certain. MAI can also be implanted at hydration levels above equilibrium, so that the buckle height will eventually decrease, slowly, after chorioretinal adhesions have occurred.[9]

MAI ABSORPTION AND RELEASE OF ANTIBIOTICS

Refojo and his coworkers[10] studied the absorption and release of antibiotics by MAI *in vivo* and *in vitro*. The implant was soaked in one of six antibiotics for one-half or one hour,

then placed on agar plates seeded with *Escherichia coli* or *Sarcina lutea*. The implants were repeatedly placed in agar plates and incubated overnight. The antimicrobial activity was assessed daily by the size of the zone of bacterial inhibition compared to zones produced by standard concentrations of the antibiotics.

With methicillin and chloramphenicol, good absorption and gradual release into the agar were demonstrated. Moderate absorption and rapid release occurred with gentamicin and polymyxin B. MAI absorbed lincomycin and bacitracin well, but their release was quite rapid.

Only bacitracin, lincomycin, and methicillin were tested *in vivo* in rabbit eyes because of the poor results with gentamicin and polymixin sulfate *in vitro* and the unlikelihood that chloramphenicol would be used prophylactically in retinal detachment surgery. Implants soaked in antibiotic solution for one hour were placed episclerally in rabbit eyes, removed three to 14 days later, and tested for antimicrobial activity. Results mimicked the studies *in vitro*. Implants soaked in methicillin exhibited antibacterial activity even after 14 days of implantation. Implants soaked in bacitracin and lincomycin showed poor antibacterial activity when they were removed from rabbit eyes after only three to five days (Figure 4).

This study showed that micropores of the implant, which resist penetration by microorganisms, absorb and retain antibiotics, and that sustained release of antibiotics at the implant site is possible.[10]

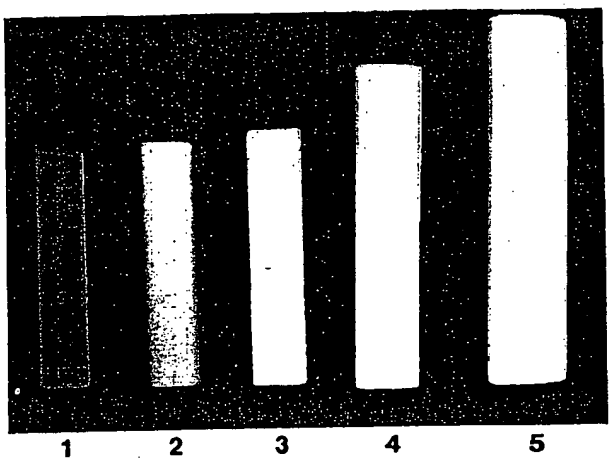


FIGURE 3: Gross picture of MAI hydrogel showing difference in size from dehydrated state (xerogel) (left) to maximally hydrated state (right). (1) xerogel; (2) implant placed in 100% ethanol and then in water; (3) placed in 30% ethanol, then water; (4) 50% ethanol, then water; (5) 70% ethanol, then water. Change in size follows graph shown in Figure 1.

ABSENCE OF TOXICITY

When a small piece of the MAI was implanted in the anterior chambers of rabbit eyes and observed for three months, no inflammatory changes were demonstrated clinically or histologically. MAI was also implanted on the episclera of rabbits and followed for three months. Again, no clinical or histological signs of significant toxicity or inflammation were demonstrated.[8]

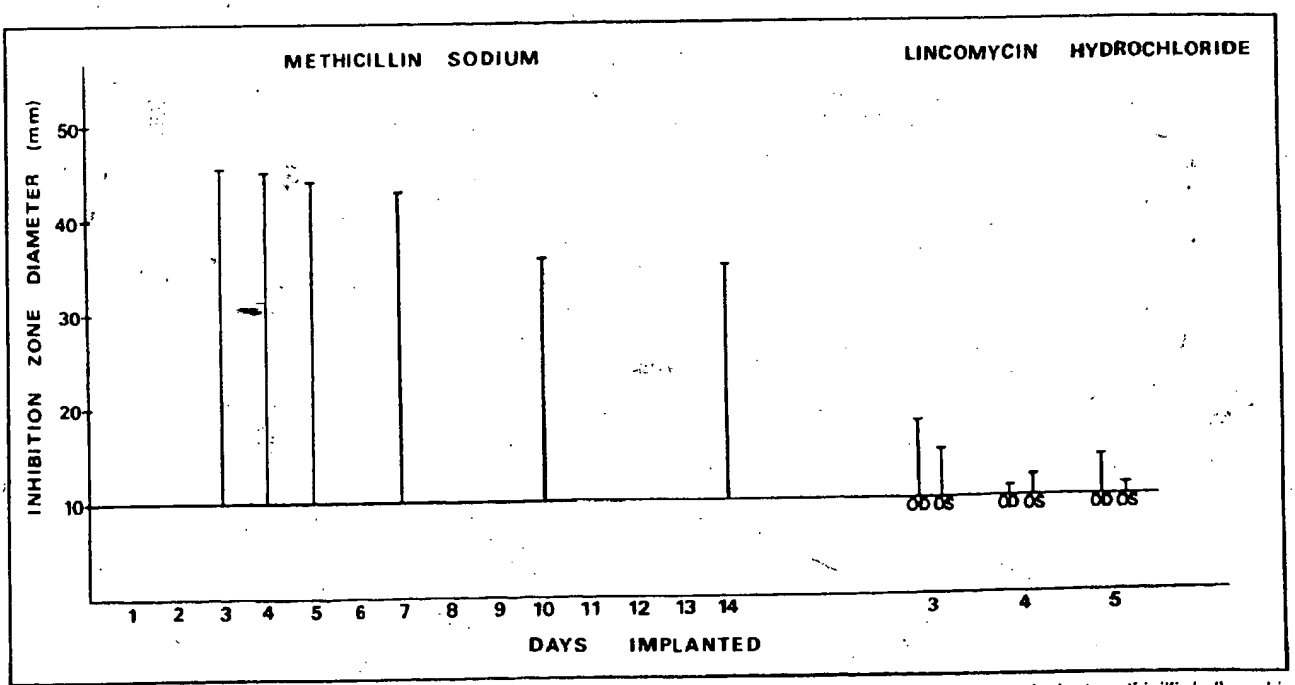


FIGURE 4: Antibacterial activity of implants (88% water) soaked for one hour in methicillin sodium (450 mg equivalent methicillin/ml) and in lincomycin hydrochloride (300 mg lincomycin/ml). Implants soaked in methicillin showed good antibacterial activity for up to 14 days, while those soaked in lincomycin showed minimal antibacterial activity after five days. Each bar represents one implant removed from a rabbit eye and tested by overnight incubation in agar plates seeded with Sarcina lutea *(10^6 colonies/ml).*

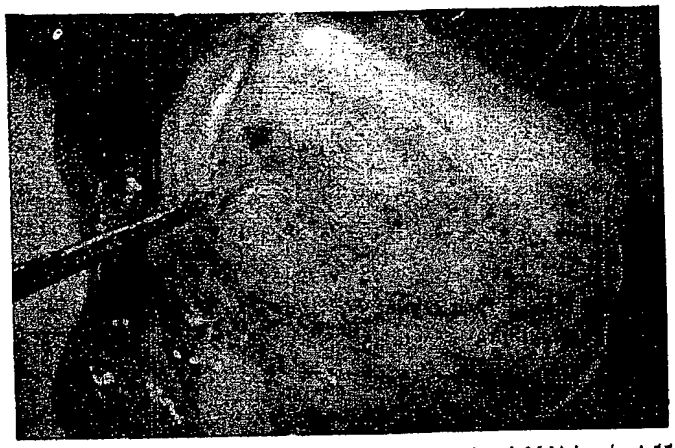


FIGURE 5: Fibrous capsule around an episcleral MAI implant 11 months after surgery on rabbit eye.

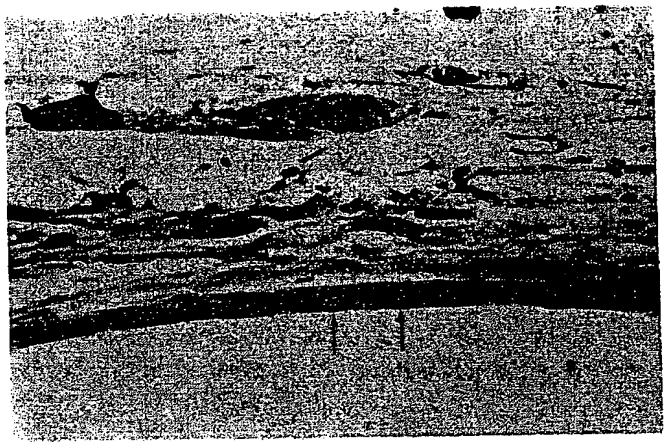


FIGURE 6A: Three months after surgery on rabbit eye with scleral dissection, an external capsule with condensation of fibers has formed in the area adjacent to the implant (arrows). Fibrovascular connective tissue is seen external to it. (Masson trichrome, original magnification × 160. (Reprinted from Retina *1983; 3:53,55).*

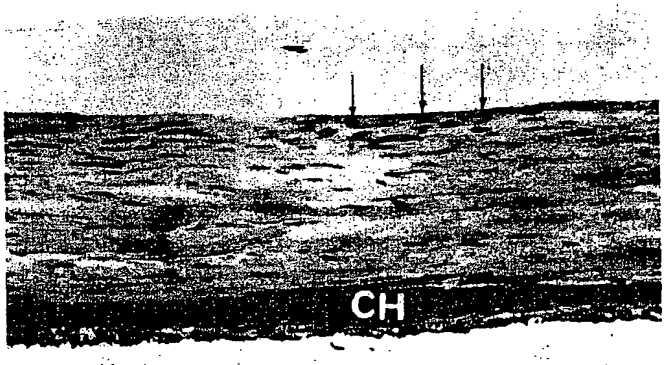


FIGURE 6B: Five months after surgery without scleral dissection, episcleral fibrous capsule is seen, with flat histiocytes and fibroblasts (arrows). Choroidal compression (CH) is noted (hematoxylin-eosin, original magnification × 160). (Reprinted from Retina *1983; 3:53,55).*

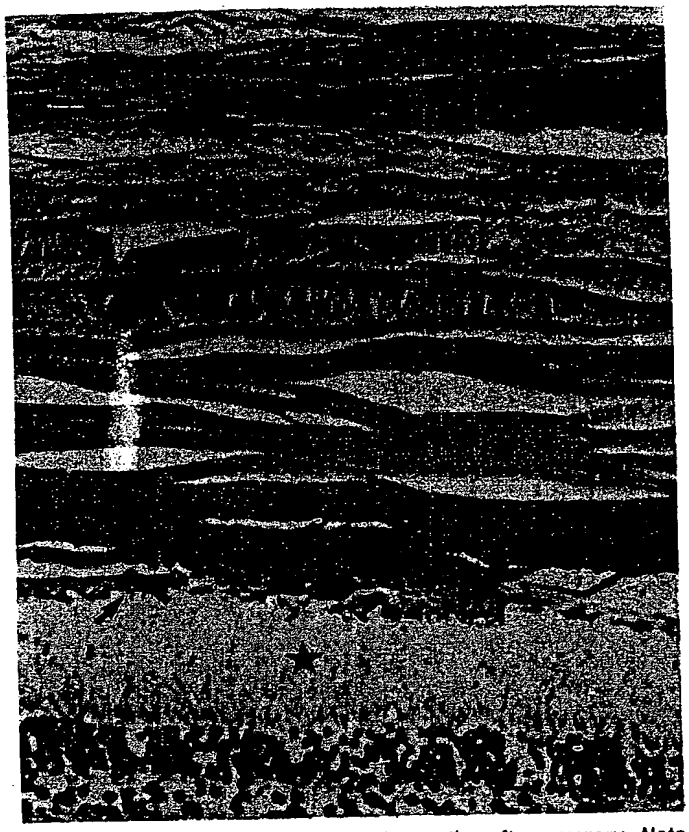


FIGURE 6C: Episcleral implant 18 months after surgery. Note acellular episcleral collagenous tissue (asterisk) next to the implant, pigment epithelial atrophy (arrow) and hypertrophy with loss of photoreceptor outer segments (star) (Masson trichrome, original magnification × 160). (Reprinted from Retina *1983; 3:53,55).*

ANIMAL TRIALS

Refojo and coworkers used MAI soaked in normal saline as episcleral and intrascleral implants in rabbit eyes. Effective buckling was achieved, with smooth buckling heights of 2 to 6 diopters during the first week of the implantation, and an average of 1.5 diopters after 20 weeks. No sign of infection, rejection, or erosion was observed.[8]

Histopathologic examination conducted two to 18 months after MAI implantation as a scleral buckle in rabbits suggested that the implant material stimulated the formation of a fibrous capsule that stabilized the implant and isolated it from the episcleral vasculature. The implants showed no gross changes in size, color, or consistency. After two to five months, the capsule contained a few condensed collagen fibers with loose vascular connective tissue external to it. In seven to 11 months after the implantation, the material was surrounded by a moderately thick, acellular fibrous capsule, 25% to 50% of the thickness of the adjacent normal sclera (Figure 5). Eleven to 18 months after the buckling, the capsule was very thick (more than 50% of the thickness of the normal adjacent sclera)[11] and acellular, with densely packed collagen fibers (Figure 6, Table).[11]

TABLE

DURATION OF FOLLOW-UP AND CAPSULE THICKNESS

Time After Surgery (mo)	Grade of Capsule Thickness* 1	2	3
2	2	—	—
	3	—	—
3	2	—	—
	2	—	—
5	1	1	—
	2	—	—
7	—	1	1
	—	1	1
11	1	1	2
	1	1	2
18	—	—	2
	—	—	2

**Grade 1, few condensed collagen fibers adjacent to the implant with loose, vascularized connective tissue external to it. Grade 2, moderately thick and poorly cellular fibrous capsule, measuring 25% to 50% of adjacent normal scleral thickness. Grade 3, thick, poorly cellular, and densely packed fibrous capsule, measuring over 50% of adjacent normal scleral thickness.*

This fibrous capsule secured the implant in position so that the buckling effect could be retained. It also strengthened the scleral bed, preventing erosion and making reoperation easy and safe.[11]

CLINICAL TRIALS

Fifty-one cases of uncomplicated rhegmatogenous primary retinal detachment were repaired using the MAI implant. Cases were followed for six to 18 months. In 25 eyes, MAI was used as meridional implant under intrascleral buckles. It was the main buckling element in 26 cases, implanted intrasclerally in 15 and episclerally in 11.[12]

The material was easy to handle during the operations. Postoperatively, effective buckle height was obtained and maintained, and no excessive inflammation was observed. In two cases in which reoperation was necessary, the fibrous capsule was smooth and easily separated from the implant.[12]

COMMENTS

MAI designed by Refojo embraces the features of an ideal buckling material: 1) It is as effective a buckling material as solid silicone rubber and silicone sponge. 2) Its softness is related to its hydration, and its degree of swelling is easily changed. 3) It is soft and elastic, thus preventing erosion. 4) It has no dead spaces, and its ability to absorb and gradually release antibiotics will probably contribute to the reduction of infections. 5) It stimulates the production of a fibrous capsule around the implant, thus strengthening the scleral bed and making reoperation easy and safe.

REFERENCES

1. Schepens CL: *Retinal Detachment and Allied Diseases.* Philadelphia, WB Saunders, 1983, pp 333-343.
2. Refojo MF: Current status of biomaterials in ophthalmology. *Surv Ophthalmol* 1982; 26:257-265.
3. Refojo MF: Sustained release of antibiotics from scleral buckling materials. II. Silicone sponge. *Ophthalmic Res* 1975; 7:459.
4. Hahn HS, Lincoff A, Lincoff H, et al: Infection after sponge implantation for scleral buckling. *Am J Ophthalmol* 1979; 87:180.
5. Russo CE, Ruiz RS: Silicone sponge rejection: Early and late complications in retinal detachment surgery. *Arch Ophthalmol* 1971; 85:647.
6. McMeel JW, Naegele DF, Pollalis S, et al: Acute and subacute infections following scleral buckling operations. (Symposium: Postoperative Endophthalmitis). *Ophthalmology* (Rochester) 1978; 85:341-349.
7. Hagler WS, Jarrett WH II, Smith JA: Infections after retinal detachment surgery. *South Med J* 1975; 68:1564-1569.
8. Refojo MF, Natchiar G, Liu HS, et al: New hydrophilic implant for scleral buckling. *Ann Ophthalmol* 1980; 12:88-92.
9. Refojo MF, Leong FL: Poly(methyl acrylate-co-hydroxyethyl acrylate) hydrogel implant material of strength and softness. *J Biomed Mater Res* 1981; 15:497-509.
10. Refojo MF, Leong FL, Chan IM, et al: Absorption and release of antibiotics by a hydrophilic implant for scleral buckling. *Retina* 1983; 3:45-49.
11. Tolentino FI, Lahav M, Refojo MF, et al: Hydrophilic acrylate scleral buckling implants in rabbits: Long-term clinicopathologic observations. *Retina* 1983; 3:50-58.
12. Tolentino FI, Refojo MF, Schepens CL: A hydrophilic acrylate implant for scleral buckling: Technique and clinical experience. *Retina* 1981; 1:281-296.

C 26

Hall A
Sunday 8:30 — 10:30 AM

Immunology & Microbiology, Physiology & Pharmacology
Drug Delivery

PGM#	BRD#	AUTHORS
181	B93	Refojo, Herrero-Vanrell
182	B94	Kunou, Ogura, Hashizoe, Honda, Ikada
183	B95	Zhu, Veloso, Leong, Geary, Dunkel, Refojo
184	B96	Chen, Jaffe, Yang, Khawly, Ashton
185	B97	Nose, Parel, Lee, Cohen, DeKosac, Rowaan, Paldano, Jallet, Soderberg, Davis
186	B98	Kochinke, Wong

NOTE: Potential conflicts of interest of presenters and authors are noted at the end of the Abstract either by "None" or with codes (see code definitions under the section "Abstract Content/Commercial Relationships/Disclosure Codes" in the front matter).

181 — B93

SUSTAINED DELIVERY OF GANCICLOVIR FROM BIODEGRADABLE POLY(DL-LACTIDE-co-GLYCOLIDE) (PLGA) MICROSPHERES
((M.F.Refojo, and R. Herrero-Vanrell)) Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA

Purpose. To develop injectable microspheres of PLGA for intraocular sustained delivery of ganciclovir in cytomegalovirus retinitis. Method. Three microsphere preparations were evaluated. T1: An emulsion of an aqueous solution of ganciclovir and PLGA in methylene chloride was then emulsified in aqueous polyvinyl alcohol. Solvent evaporation and stirring yielded the microspheres. T2: Ganciclovir and PLGA in dimethyl formamide emulsified in castor oil, sesame oil, or silicone oil (SIO) using various emulgents, stirred under solvent evaporation conditions. T3: A dispersion of ganciclovir in fluorosilicone oil (FSIO) was further dispersed in a PLGA-acetone solution and then emulsified in SIO. Poly(dimethylsiloxane ethylene oxide-co-propylene oxide) was the emulgent. Stirring and solvent evaporation yielded the microspheres. Results. The best ganciclovir loading in the microspheres prepared by T1 was 60% of theoretical, and the best *in vitro* ganciclovir release was obtained with the 212-300 µm microspheres of 0.65 dl/g viscosity PLGA. Ten mg of these microspheres released 0.25 µg/h ganciclovir for 10 days, which is below the therapeutic range (~0.5-2.88 µg/h) in human eyes. In T2 the polymer precipitated and yielded negligible amounts of microspheres. The best overall drug loading was in microspheres prepared by T3: 90-95% of theoretical in 300-500 µm microspheres of 0.39 dl/g PLGA. Ten mg of the microspheres released ganciclovir *in vitro* at ~0.6 µg/h for the initial 25 days, and ~0.4 µg/h by the 42th day. Conclusions. T3 resulted in higher ganciclovir loading and prolonged the drug release compared to the microspheres prepared by standard techniques T1 & T2. T3 microspheres are under evaluation in an animal model of cytomegalovirus retinitis.
NIH grant EY-00327 (MFR) and a NATO grant (RH-V). P

182 — B94

BIODEGRADABLE SCLERAL IMPLANT FOR INTRAOCULAR CONTROLLED DELIVERY OF CORTICOSTEROID
((N. Kunou[1], Y. Ogura[1], M. Hashizoe[1], Y. Honda[1], and Y. Ikada[2]))
Department of Ophthalmology[1], and Research Center for Biomedical Engineering[2], Kyoto University, Kyoto, Japan

Purpose. The authors evaluated the feasibility of poly(D,L-lactide-co-glycolide) (PLGA) scleral implant as long-term intraocular controlled delivery of betamethasone sodium phosphate (BP) for the treatment of chronic uveitis.
Methods. The scleral implants containing BP were made of PLGA (ratio: 85/15, MW: 86000). The implants were prepared by compression molding of the homogeneous BP/polymer mixtures pre-treated with freeze-drying of BP/polymer solution. The 10 and 25% BP-loaded implants were prepared and investigated as follows. The release of BP from the implants were evaluated in 0.1M phosphate buffer solution at 37 °C. The devices were implanted at pars plana in pigmented rabbits eyes. BP concentrations in the aqueous humor, vitreous and retina/choroid were determined by HPLC. Toxicity was evaluated by scotopic ERGs.
Results. Biphasic release patterns were observed in *in vitro* release test and BP was released over one month. After initial burst, the drug was released more slowly. BP concentrations in the retina/chorid after implantation of both 10 and 25% loading were maintained in the range of effective concentrations suppressing inflammation processes over one month. On the other hand, BP in the aqueous humor was below detection limits. BP release from the implants in the eyes was correlated with that *in vitro*. BP release was completed before beginning of PLGA weight loss in the eyes and dependent on water uptake into the implants. No toxic reactions were not observed.
Conclusions. Our findings suggested that the PLGA scleral implant may be a promising device for the controlled intraocular delivery of BP.
P

183 — B95

EFFICACY OF BIODEGRABLE MICROSPHERES LOADED WITH GANCICLOVIR DURING CYTOMEGALOVIRUS (CMV) INFECTION ON HS68 MONOLAYERS. Q. Zhu, A. Veloso, FL. Leong, P. Geary, E. Dunkel, MF. Refojo, Schepens Eye Research Institute, Harvard Medical School, Boston, MA.

Purpose: Intravitreal ganciclovir (DHPG) is effective in delaying the progression of CMV retinitis in patients with AIDS. However, multiple injections are associated with an increased risk of endophthalmitis, retinal detachment and vitreous hemorrhage. To decrease risk and to provide a longer therapeutic effect, Refojo and Herrero-Vanrell (ARVO 1995) developed biodegrable poly (DL-lactide-co-glycolide) (PLGA) microspheres loaded with DHPG. This study evaluated the efficacy of the microspheres to treat CMV infection in Hs68 monolayers. Methods: 300-500um microspheres were prepared from 250mg of 0.39dl/g PLGA and 25mg of DHPG. Six groups of microspheres, each group included 1, 0.5, 0.25 and 0.1mg of microspheres respectively, were submerged in DMEM medium separately. Cytopathic effects (CPE) inhibition assay was performed weekly on Hs68 monolayer onto which CMV was inoculated and with which microspheres were co-cultured. The efficacy is presented as reduction in CMV CPE compared to non-treated CMV CPE values. Viral quantitation inhibition assay was performed. After 48-60 hours inhibition co-culture of microspheres, the monolayer was harvested and virus was titrated for the quantitation. The effect dose values in time course was determined by the quantity of inhibited viral titer. Results: Microspheres had no toxic effect on the Hs68 monolayer. In the first two weeks, on all doses of microspheres, CMV CPE was totally inhibited. From the third to fifth week, CPE reduced 50-86%. By the last week, CPE reduced 47-36% compared to non-treated controls. Viral quantitation inhibition assay indicated that 1mg/ml of microspheres inhibited more than two digit of virus titer during 6 weeks; during the first three week, was superior to, and in the last three week, was similar to 3ug/ml of DHPG for inhibition of CMV titer. Conclusion: Microspheres loaded with DHPG provided an effective sustained delivery of DHPG for CMV infection treatment in vitro, and suggested that 1mg/ml of microspheres can be used as the dosage for in vivo treatment. Supported by EY00327(MFR), N0I-AI-15100(ECD) and P(MFR)

184 — B96

DEVELOPMENT OF A 1.5 YEAR GANCICLOVIR IMPLANT FOR CMV RETINITIS ((J. Chen[1], G.J. Jaffe[2], D.C.S. Yang[2], J.A. Khawly[2] and P Ashton[1].
1 New England Eye Center, Boston MA. and 2 Duke Eye Center Durham NC))

Purpose: To develop an implantable device giving sustained release of ganciclovir (GCV) over 1.5 years. Methods. Short rod like pellets of GCV were prepared by compressing 30 mg of GCV in a 2 mm tablet die. Pellets were then coated in a solution of 98% hydrolyzed polyvinyl alcohol (PVA). This polymer dries to give a tough, non-erodible yet permeable coating. The pellets were then partially coated in films of the impermeable polymer ethylene vinyl acetate leaving a diffusion "port" to allow release. The assembly was then coated in PVA and heat treated to further control the release rate. Release rates were determined by immersing pellets in 5 ml of phosphate buffer (pH 7.4) and periodically sampling. Release studies were continued for up to 60 days with replacement of the receptor solution every 5-6 days. Analysis was by HPLC. Concurrent with the release work, an investigation of optimal position of suture strut was performed using human cadaver eyes. Results: Release rate was found to be primarily a function of the size of the diffusion port and alterations in the heat treatment or the use of PVA as a binder in the initial tableting step could provide further "fine" control (a factor of 2-4). Optimization allowed devices to be prepared releasing GCV at 1-2 ug/hr over the 60 day testing period. These devices have a theoretical duration of over 600 days. Work with cadaver eyes indicated that the rod style of device can be expected to be readily implantable in human eyes. Conclusion: Implantable devices releasing GCV at 1-2 ug/hr have previously been found to effectively control CMV retinitis in man with progression occurring in newly diagnosed patients only after device depletion (1). Because of the increasing lifespan of patients with AIDS, the 600 day implant described here may represent an advantage over existing systems.

1) Martin et al . Arch Ophthalmol. 112:1531-1539 (1994).
Supported by EY 09973 and Research to Prevent Blindness, E (3)

185 — B97

OCULAR COULOMB CONTROLLED IONTOPHORESIS (OCCI)
((I. Nose[1], J-M. Parel[1,2,3], W. Lee[1], F. Cohen[3], Y. DeKosac[3], C. Rowaan[1], A. Paldano[1,2], V. Jallet[1,2], P. Söderberg[1], J. Davis[1]))
Bascom Palmer Eye Institute[1] and Dept of Biomedical Engineering, University of Miami, FL[2]; University of Paris Hôtel Dieu Hospital and INSERM U86 & 112, France[3].

Purpose. Develop and test an ocular drug transfer system (OCCI) based on Coulomb's laws to obtain reliable intraocular drug concentration in a short time while avoiding tissue damage. Methods. A programmable 3 microcomputer chips electronic system was designed to produce an equipotential electrical field with a continuously monitored adjustable constant direct current source connected to 2 electrodes. The active ocular electrode has a constant liquid surface and a drug reservoir connected to 2 syringes containing the drug solution and a partial vacuum. A very low impedance return electrode is used. Transcorneal, transscleral and transcorneoscleral OCCIs were made for experimentation in rabbits and rats. The drug solutions were modified for optimal iontophoretic transfer. Results. No extra- or intraocular tissues damage was detected clinically or by light microscopy. A ≥0.08C/cm² transcorneal transfer of pimaricin produced 3 MMC in 4min. A ≥500µg/ml Di-hematoporphyrinether intracorneal concentration was obtained for PDT treatment of acanthamoeba infections. Higher intravitreal concentration of fluorescein and dexamethasone were obtained. Conclusion. This pilot study demonstrates OCCI's safety and effectiveness with 4 drugs. Ganciclovir and other drugs are under study. Pharmacokinetics and TEM analyses and extended in vivo studies are necessary before using OCCI in clinical trials.

Supported by the Florida Lions Eye Bank, Miami Veterans Administration Hospital and Research to Prevent Blindness, New York, NY. None.

C27

Jpn. J. Ophthalmol.
Vol. 28: 140-146, 1984

A METHOD FOR CHRONIC DRUG INFUSION INTO THE EYE

Koichiro MIKI, Hiroshi OHKUMA and Stephen J. RYAN

Summary: **A method was established for chronic drug delivery into the vitreous of the rabbit eye. A cannula was inserted into the eye through the pars plana. A silicone plate controlled the intraocular location of the tip of the cannula; this plate also served as an anchor plate to the sclera. The distal end of the cannula was attached via a larger diameter silicone tubing to an implanted osmotic mini-pump. Cannulas remained patent and there was almost no evidence of ocular trauma after one year follow-up. This method thus has a potential use for experimental studies involving chronic application of drugs.**

Key Words: *Rabbit, intraocular implantation, silicone tube, osmotic pump*

Introduction

Drug infusion into the eye is often required in experimental ocular studies. Injection through the pars plana is the usual route of administration[1,3,4,7,8]. However, continuous infusion is desirable to maintain a high level of drug concentrations. Previous investigators[5,6] have utilized a continuous intraocular infusion system using an osmotically driven mini-pump. However, the methodology of cannula implantation and its ocular effects were not described in detail.

In this paper we describe the surgical technique of intraocular implantation of a long-term indwelling cannula. Pathological findings in the cannulated eyes will be reported in a subsequent paper.

Materials and Methods

1. *Implants*

The cannula consisted of approximately 50 mm of silicone tubing (silastic tubing No. 602-105, Dow-Corning), chosen because of its softness and pliability[9]. The intraocular portion of the tubing (approximately 7.5 mm in length) was angled at 135 degrees to the extraocular portion (Figure 1A). This directed the tip toward the posterior fundus of the rabbit eye. A 7 mm silicone plate fixed the angle and allowed an accurate fixation of the cannula to the sclera. In five animals, the extraocular end of the cannula was connected by an external silicone tubing (silastic tubing No. 602-155, Dow-Corning) to an Alzet osmotic mini-pump (Model 2001 or 2002, Alza Corp, Stanford, CA) (Figures 1A and B).

2. *Experimental procedures*

Fifty-six adult pigmented rabbits weighing 2 to 3 kg were used. Animals were anesthetized

Received: January 18, 1984
Department of Ophthalmology, University of Southern California School of Medicine, and the Estelle Doheny Eye Foundation, Los Angeles, California
Reprint requests to: Stephen J. RYAN, M.D., Department of Ophthalmology, USC School of Medicine, Estelle Doheny Eye Foundation, 1355 San Pablo Street, Los Angeles, CA 90033, USA

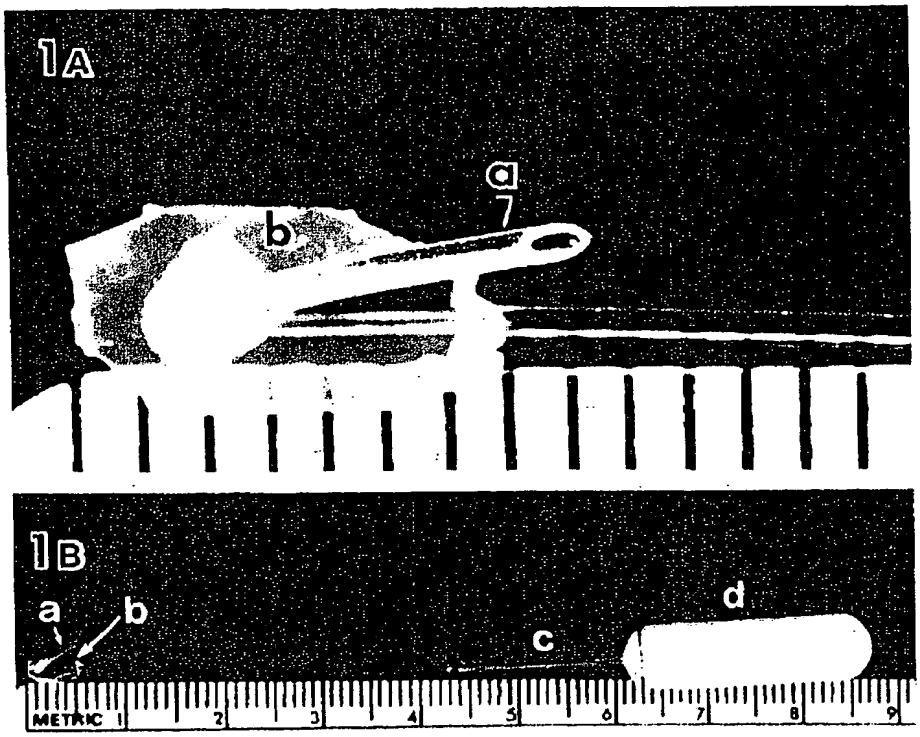


Figure 1A. High magnification of intraocular cannula and attachment plate. Each division represents 1 mm. Intraocular portion of cannula is fixed by plate at 135-degree angle to extraocular portion of tubing.

B. Chronic drug delivery system
   a. intraocular portion
   b. plate
   c. connecting tubing
   d. pump

with a mixture of ketamine hydrochloride, 20 mg per kg, and xylazine hydrochloride, 10 mg per kg, injected intramuscularly. The eyes were dilated with 1% tropicamide. A skin incision was made in the upper orbital edge with a number 11 Bard-Parker blade and the upper orbital septum was opened at its base. A conjunctival flap was turned anteriorly to the upper limbus and the eye was stabilized with a 4-0 silk bridle suture on the superior rectus muscle. After cauterizing the bleeding points in the wound, a one mm radial scleral incision was made with a number 11 Bard-Parker blade one mm posterior to the limbus and two mm temporal to the superior rectus muscle. Prior to insertion of the cannula, the choroid and the dense vitreous were cut with a 22-gauge needle, which was guided to the middle of the vitreous cavity without touching the lens, thereby creating a track for the cannula.

A small amount of vitreous was lost from the scleral wound when the needle was removed from the eye. Speedy introduction of the cannula minimized additional vitreous loss. The cannula was advanced under funduscopic control until its tip reached the posterior vitreous, close to but not touching the retina (Figure 2). The cannula plate was then firmly fastened to the sclera by 9-0 nylon sutures to prevent withdrawal from the vitreous cavity or retinal contact. The Tenon's capsule was drawn over the plate and sutured with the 9-0 nylon sutures. The external

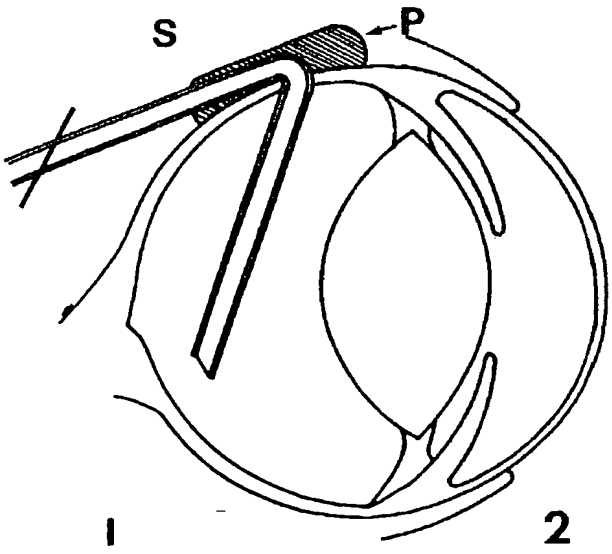


Figure 2. Diagram of cannulated eye.
S. Superior
P. Plate
I. Inferior

end of the cannula was carried through the opened orbital septum and through the skin wound. The orbital septum was then closed with 9-0 nylon sutures. The skin wound was closed by 4-0 silk sutures. The external end of the cannula was knotted to prevent invasion by wound-healing tissue or foreign organisms, and anchored to the skin by 4-0 silk sutures.

In five animals, 1-2 months after the implantation, 10 μl of sodium fluorescein in balanced salt solution (1:150) was injected into the eye through the cannula to demonstrate its patency. The intraocular portion of the cannula was inspected for the appearance of the dye with the ophthalmoscope and/or documented by a Zeiss fundus camera. In another five animals, a mini-pump filled with diluted fluorescein was attached to the external end of the cannula and placed beneath the forehead skin at the time of cannulation.

3. *Postoperative management and course*

Following implantation, 1% atropine sulfate ointment and bacitracin-neomycin-polymyxin ointment were applied daily for three days. At the same time, intramuscular gentamicin sulfate (20 mg) and methicillin sodium (250 mg) were given. The eyes were examined periodically with the indirect ophthalmoscope and slit-lamp biomicroscope. Fundus photographs were also taken. The intraocular pressure was measured by Schiøtz tonometry on 16 rabbits at various times from one week to one year after implantation.

Table 1. Observation period in 32 eyes with successful implantation

Number of eyes	Period
10	1- 4 weeks
3	1- 2 months
4	2- 3 months
5	3- 6 months
10	6-12 months

Results

Successful implantation was obtained in 32 of the 56 eyes. Table 1 shows the duration of observation following the 32 implantations.

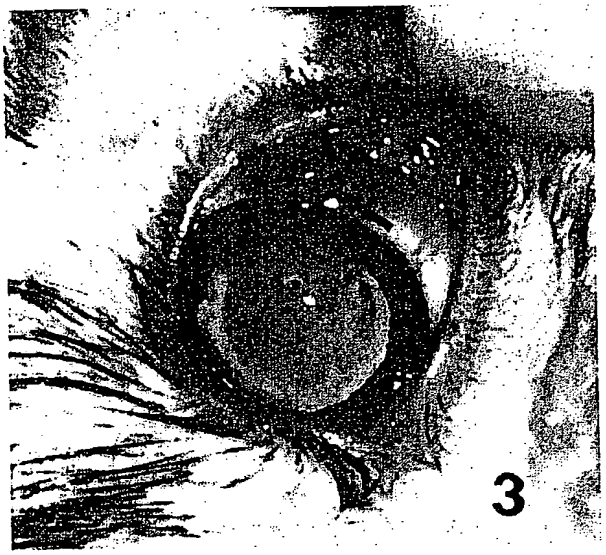


Figure 3. Anterior segment immediately after surgery. Slight irritation is visible around cannula plate (indicated by arrow).

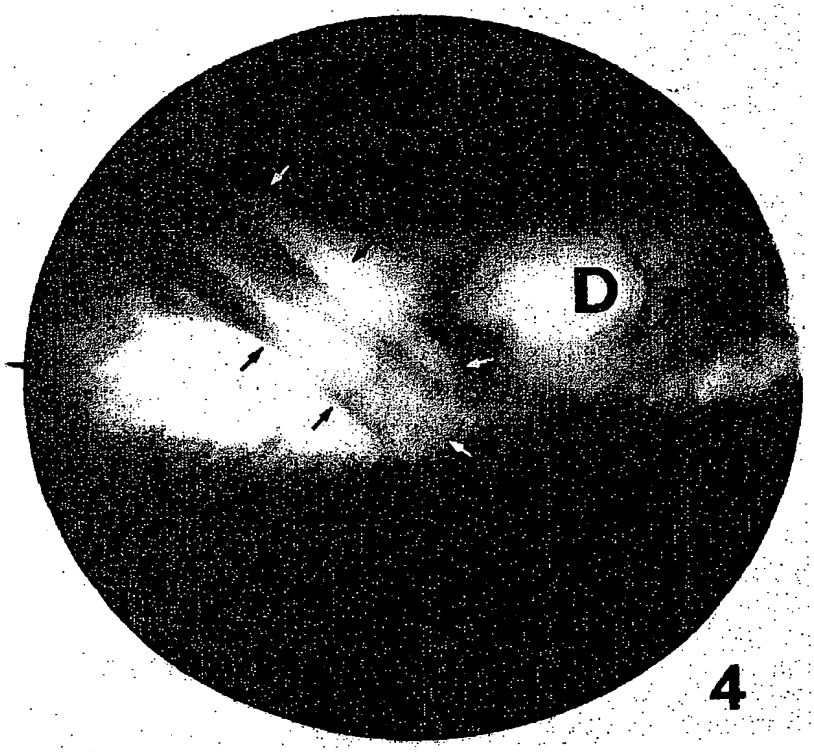


Figure 4. Ocular fundus and cannula (arrows) one year following implantation. Cannula appears out of focus because it is seen through vitreous. D: Optic disk

1. *Clinical course*

During the first week after implantation, all eyes exhibited moderate irritation of the anterior segment, eg, a very slight iritis and mild scleral inflammation around the site where the cannula sleeve was located, and conjunctival injection, particularly over the cannula plate. A portion of the cannula plate was visible through the conjunctiva (Figure 3). At least two weeks were required for these changes in the anterior segment to subside, after which all eyes remained quiescent. Immediately after the operation, a small vitreous hemorrhage around the basal part of the cannula occurred in eleven eyes. No additional vitreous hemorrhage was noted throughout the postoperative course. The cannula tip in each eye appeared clear, and was located in the posterior vitreous just anterior to the optic disk (Figure 4). The difference in intraocular pressure between the implanted and the fellow eye was within 0.6 mm Hg and remained stable during the follow-up.

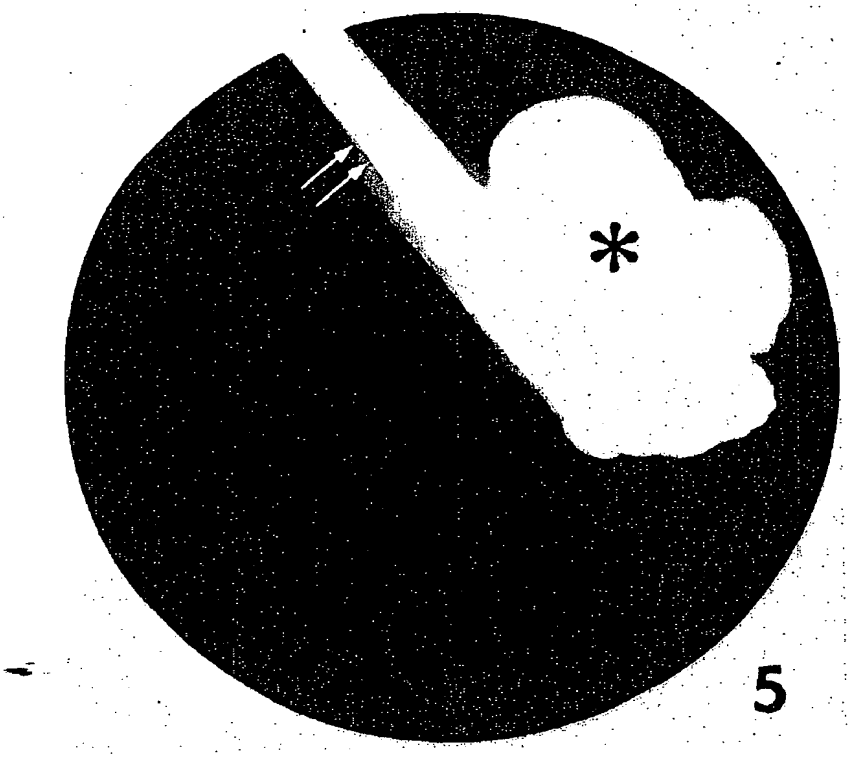


Figure 5. Diluted sodium fluorescein, injected through cannula, is visible in vitreous (*) as well as in cannula (double arrows).

Table 2. Complications in 24 unsuccessfully cannulated eyes

Type of complication	Number of eyes	Time of occurrence
Infection	6	1-15 weeks after implantation
Implant extrusion	6	1-11 weeks after implantation
Lens damage	3	Intraoperative
Retinal touching and tearing	5	Intraoperative
Traction retinal detachment	4	5-23 weeks after implantation

2. *Cannula patency*

One to three months after the implantation, diluted fluorescein was injected by microsyringe into the external end of the cannula in five rabbits. Fluorescein diffused immediately into the vitreous (Figure 5), proving patency. The five eyes with the mini-pumps containing fluorescein also demonstrated cannula patency. The details about the function of this system will be presented in a subsequent report.

3. *Complications*

Complications which rendered 24 of the 56 implantations unsuccessful are summarized in Table 2. During the development of our method, the main causes of failure were conjunctival tearing over the cannula plate, which may have enhanced the risk of invasion of organisms from the conjunctiva, and a gap between the plate and the sclera, both of which were followed by slippage and rejection of the cannula.

Lens opacities occurred when the cannula tip scratched the lens capsule. Retinal damage occurred when the cannula touched the retina due to excessive pressure on the plate or too much maneuvering of the cannula during implantation. These problems were avoided with experience. In some animals a strand was seen between the wound and the surface of the retina along the cannula. The strand selectively involved the area of the optic disk and the medullary rays and caused focal traction retinal detachment. This complication resulted from the medullary rays being touched by an excessively long cannula during the insertion. Also, if the angle of the tubing were too acute, constant contact of the cannula tip with the retina could occur. Moderate vitreous hemorrhage or chronic inflammation also contributed to the development of fibrovascular traction bands. In later experiments, the length of the cannula was reduced from 9 mm to 7.5 mm and the angle to the sleeve was set at 135 degrees; this prevented these complications.

Discussion

In our initial studies, 20 of 38 eyes developed various complications. The problems experienced were eventually eliminated by refinement of the surgical techniques. After the evolution of a standard approach and methodology, and with the acquisition of improved surgical skills, only 4 of 18 eyes developed complications. At present, the implantation technique, properly performed, does not appear to adversely affect the eye. It is important to emphasize that strand formation between the cannula and the retina, which routinely results in traction retinal detachment, developed in only 4 eyes operated on early in the experiment. It has not been a problem since the procedure has been perfected. This complication occurred typically between the 5th and 23rd week after implantation, when chronic eye inflammation or moderate vitreous hemorrhage occurred[2,10].

In conclusion, the present study is a first step in the development of an intraocular chronic drug delivery system in the rabbit. The results indicate that most of the eyes were resilient enough to withstand the implantation, although studies of ocular histopathology will be needed before it is possible to exclude subtle changes caused by cannulation. It is hoped that this implantation technique will offer a convenient tool for future studies involving the treatment and pharmacologic manipulation of various intraocular conditions, such as massive periretinal proliferation and subretinal neovascularization.

Acknowledgments: This study was supported in part by Grants EYO1545 and EYO3040 from the National Institutes of Health.

We wish to thank Ann Dawson for editorial assistance, Sue Gertson for typing assistance and Randi Patterson for photographic assistance.

The animals used in this study were maintained in animal care facilities fully accredited by the American Association of Laboratory Animal Science.

REFERENCES

1. Burke J M & Smith J M: Retinal proliferation in response to vitreous hemoglobin or iron, Invest Ophthalmol Vis Sci 20: 582-592, 1981
2. Cleary P E & Ryan S J: Histology of wound, vitreous, and retina in experimental posterior penetrating eye injury in the rhesus monkey, Am J Ophthalmol 88: 221-231, 1979
3. Cobo L M & Forster R K: The clearance of intravitreal gentamicin, Am J Ophthalmol 92: 59-62, 1981
4. Daily M J, Peyman G A & Fishman G: Intravitreal injection of methicillin for treatment of endophthalmitis, Am J Ophthalmol 76: 343-350, 1973
5. Eliason J A & Maurice D M: An ocular perfusion system, Invest Ophthalmol Vis Sci 19: 102-105, 1980
6. Michelson J B & Nozik R A: Experimental endophthalmitis treated with an implantable osmotic mini-pump, Arch Ophthalmol 97: 1345-1346, 1979
7. Peyman G A: Antibiotic administration in the treatment of bacterial endophthalmitis, II Intravitreal injections, Surv Ophthalmol 21: 332-346, 1977
8. Salminen L, Fischer W, Jantti V & Weigelin E: Rate of loss of intravitreally injected fluorescein and EDTA in the rabbit eye, Ophthalmic Res 13: 60-64, 1981
9. Schocket S S, Lakhanpal V & Richards R D: Anterior chamber tube shunt to an encircling band in the treatment of neovascular glaucoma, Ophthalmology 89: 1188-1194, 1982
10. Topping T M, Abrams G W & Machemer R: Experimental double-perforating injury of the posterior segment in rabbit eyes, The natural history of intraocular proliferation, Arch Ophthalmol 97: 735-742, 1979

EXPRESS MAIL MAILING LABEL
NO. EV219067000US

C28

XP-002279551

(C) MEDLINE / NLM
AN - NLM1763453
AU - Nesterov A P; Basinskii S N
DT - Journal Article
IRN - ISSN 0042-465X
LA - rus
TFT - IM
XNPL- 0042-465X-107-5-49
AB - A new method for instillation and deposition of drugs in the sub-Tenon's space has been developed, that permits the maintenance of a high concentration of the drug directly in contact with the posterior segment sclera and membranes of the optic nerve anterior segment. It consists in the following: a collagen hemostatic sponge, connected to a silicone tube, is implanted into the sub-Tenon's space; the drugs are administered via this tube. Analysis of the results of 115 surgeries has demonstrated considerable advantages of this method over the traditional methods of drug instillation.
COJ - 0415216
DCOR- 2001-11-02
IW - English Abstract; Evaluation Studies; Glaucoma: surgery; Human; Infusion Pumps, Implantable(#); Ophthalmologic Surgical Procedures(#); Trabeculectomy
NR - 5
OT - Novyi metod vvedeniia lekarstvennykh preparatov v zadnii otdel subtenonova prostranstva.
PD - 1991-09-00
PG - 49 - 51
PUB - Vestnik oftalmologii.
- USSR
- 1991 Sep-Oct
TI - [A new method of drug administration into the posterior section of the sub-Tenon's space]
VOL - 107
AUW - Nesterov A P; Basinskii S N